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Response of *Tagetes patula* L. and *Ageratum houstonianum* Mill. to Microbial Biostimulant Inoculation and Organic Fertilization

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Abstract: A correct cultivation technique supported by scientific evidence that leads to high-quality standards can promote sustainable floriculture. It is urgent to find alternative solutions to the widely used chemical fertilizers and evaluate the effectiveness of other fertilizers. The liquid organic ones, already in use in organic vegetable farming, could be a good substitute if supplied together with growth-promoting products such as microbial biostimulants. In the hope of replacing the traditional chemicals with a more sustainable organic-based fertilization, the present investigation aimed to evaluate the effects of a microbial biostimulant and various combinations of organic and mineral fertilization on morphological characteristics and physiological parameters of *Tagetes patula* L. and *Ageratum houstonianum* Mill. The plants were grown in pots with a substrate inoculated or not with the microbial biostimulant and were fertigated with nutrient solutions at different concentrations of elements from mineral and/or organic sources. Six fertilization formulas were adopted: control (only water without fertilizer), 100% mineral fertilization, 50% mineral fertilization, 100% organic fertilization, 50% organic fertilization, and 50% mineral + 50% organic fertilization. For the organic fertilization, a commercial liquid fertilizer admitted in organic farming with 3-2-5.5 NPK with 3% organic nitrogen was used. Mineral fertilization was formulated to match the organic solution as closely as possible. We observed an improvement in ornamental value (stem diameter and shoot number) with the biostimulant inoculum. Generally, the 50% mineral and 50% organic fertilization did not negatively influence the morphological characteristics. The reduction by 50% in the mineral nutrients and the integration of this reduction with an organic fertilizer was feasible to produce potted plants of these species during spring in the Mediterranean area.

Keywords: bedding plants; French marigold; flossflower; liquid fertilizer; cultivation technique; organic farming; sustainable floriculture; plant growth-promoting microorganisms

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1. Introduction

Today, there is an urgent need for an ecological transition that involves every productive sector. Sustainable and environmentally friendly production systems are widely studied in agriculture. Within this sector, floriculture is the production system probably less involved in these studies and more difficult to convert into sustainable growing systems. Some authors investigated the awareness of flower and ornamental plant consumers of the environmental sustainability of floriculture and their willingness to pay (WTP) for environmentally friendly products. The results highlighted that WTP increases for plants labeled non-invasive, native, or sold in biodegradable or low-carbon packages [1]. Environmentally friendly products have an intangible added value linked to their quality and respect for the environment. Sustainable production is a concrete opportunity for floriculturists that aim to diversify the quality level and widen the types of their products.

Wholesaler, retailer, and consumer demand have begun pressuring the floriculture industry to become more sustainable [2]. The demand for organic and sustainable flower products is increasing in the USA as a result of an emerging market segment focused on health and fitness, environment, personal development, sustainable living, and social justice, known as "lifestyles of health and sustainability" (LOHAS) [2,3]. Already in 2005, the organic flower market was the fastest-growing sector of the nonfood organic market in the USA.

Sustainable floriculture production aims to reduce environmental degradation [4], maintain productivity, promote economic viability, conserve resources and energy, and maintain stable communities and quality of life [5]. Sustainable practices include recycling irrigation water and plastic, implementing biological control, using alternative energy sources [6] and reducing chemical inputs. The barriers that limit the spread of sustainable floriculture include the cost of technology, the lack of economic incentives, and also the age, the level of education, the risk perceptions, and the lack of knowledge of the floriculturists [7–12]. Floriculture is an agricultural sector that produces goods with very high-quality standards, considered discretionary, and destined for a market where appearance and perfection must be at the highest level. Sustainable floriculture can be a new commercial segment where it is essential to identify, from a scientific point of view, sustainable cultivation techniques able to provide high-quality products. Ornamental plant growers are often compelled to use chemical input in order to produce high-value products that can allow appropriate incomes [5]. Nevertheless, it would be wise to find alternative solutions to chemical fertilizers and evaluate the effectiveness of other fertilizers, such as the organic fluid fertilizers already used in organic vegetable farming. In this crucial phase of ecological transition, plant growth promoters such as biostimulants may be beneficial. Microbial biostimulants are commercial products containing microorganisms such as mycorrhizal or non-mycorrhizal fungi and bacteria able to colonize the rhizosphere and improve plant growth through multiple mechanisms, such as soil aggregation [13], nutrient solubilization and mobilization [14,15], control or suppression of some root pathogens [16,17], and increase in plant tolerance to abiotic stresses [18,19].

Some ornamental pot species, such as *Petunia hybrida* and *Chrysanthemum morifolium*, showed positive effects of microbial biostimulant application on plant growth and yield [20,21]. Nevertheless, the research on the response of ornamental species to organic fertilization and plant growth-promoting microorganisms is still limited, and even widely spread species, such as *Tagetes patula* and *Ageratum houstonianum*, still need to be studied to increase the sustainability of their production systems. *Tagetes* and *Ageratum* belong to the *Asteraceae* family and are annual herbaceous species widely used as bedding plants [22]. The aerial parts of both plants contain high-quality essential oils used in perfume soaps, perfumery, cosmetics, and pharmaceutical industries [23]. Furthermore, *Tagetes* is a precious crop for nematode management [24–27], while *Ageratum* is appreciated for its antibacterial activity [23,28]. The use of microbial biostimulants in combination with organic fertilizers may help improving the sustainability of these crops. *Tagetes* plants have been found to benefit mycorrhizal fungi inoculation under drought stress [29], while there is still no study on the effects of plant growth-promoting microorganisms on *Ageratum* plants. Due to the shortage of literature reports on these species about the combined use of microbial biostimulants with organic fertilizers to reduce the chemical fertilization and increase the sustainability of bedding plant crop systems, the present investigation aimed to assess the effects of a microbial biostimulant inoculum and various combinations and levels of organic and mineral fertilizers on morphological characteristics and physiological parameters of *Tagetes patula* L. (French marigold) and *Ageratum houstonianum* Mill. (flossflower).

2. Materials and Methods

The research was carried out at the Department of Agricultural, Food, and Forest Sciences (University of Palermo, Italy) on fixed benches of a high shaded greenhouse

(38°6′28″ N 13°21′3″ E; altitude 49 m). Seeds of *Ageratum houstonianum* Mill. (Vilmorin, La Ménitré, France) and double dwarf *Tagetes patula* L. (Blumen, Piacenza, Italy) were sown on 24 April 2021 into plastic pots of 8 cm diameter (207 cm^3) filled with a commercial substrate (Utilis, GreenView srl, Crocetta del Montello, Italy) composed of a mixture of slightly and fully decomposed raised bog peat (pH 6.0—electrical conductivity EC 0,20 dS/m; containing 850 g m^{-3} of a mineral fertilizer NPK 12-11-18), or with the same substrate inoculated with 0.75 g L^{-1} of Flortis Micorrize (Orvital, Settimo Milanese, Italy) [18]. This commercial biostimulant contains 30% of *Glomus*, *Rhizophagus* and *Funneliformis* spp., 1.24 × 10^8 CFU g^{-1} of *Agrobacterium radiobacter*, *Bacillus subtilis*, *Streptomyces* spp. and 3 × 10^5 CFU g^{-1} of *Thricoderma* spp. After sowing, the pots were watered, kept in a dark room at 25 ± 1 °C, and transferred to the greenhouse for plant growth as soon as the first plantlets emerged. Five days after emergence, the plantlets were thinned to leave one plant per pot.

During plant growth, the irrigation water and the nutrient solutions were supplied uniformly to all the pots through an ebb-and-flow system. Plants were fertilized once a week. Three replicates of 5 pots for each treatment were randomly arranged on a bench in a factorial design in which plants inoculated (M+) and non-inoculated (M−) with microbial biostimulant were fertigated with six nutrient solutions (NSs) with different concentrations of elements from mineral and/or organic sources: control (only water without fertilizer), NS with 100% mineral fertilizers (100% MF), NS with 50% mineral fertilizers (50% MF), NS with 100% organic fertilizers (100% OF), NS with 50% organic fertilizers (50% OF), and NS with 50% mineral fertilizers and 50% organic fertilizers (50% MF + 50% OF). To prepare the organic nutrient solution, a commercial fluid fertilizer (Geolia, Weldom-Breuil le Sec 60608 Clermont Cedex France) was used. It had 3-2-5.5 NPK with 3% total organic nitrogen deriving from sugarcane, organic fish flour and wheat plant residues. The 100% organic nutrient solution was prepared by adding 4.5 g L^{-1} of the organic fertilizer to the irrigation water, resulting in 135, 90 and 248 mg L^{-1} of N, P_2O_5, and K_2O, respectively. The mineral nutrient solutions were formulated to match the organic nutrient solutions as closely as possible using water-soluble mineral fertilizers [30]. The nutrient solutions had an EC that ranged from 1.6 (100% M) to 1.9 (100% O) mS cm^{-1} and a pH ranging from 6.8 to 6.3.

The cultivation period, from sowing to flowering, lasted about 2 months. During the crop cycle, the air temperature and the relative humidity in the greenhouse were monitored. The average temperature remained around 20 °C until the end of May (Figure 1), whereas the relative humidity was 73.7± 1.5% on average, and ranged between 26.1% and 100%.

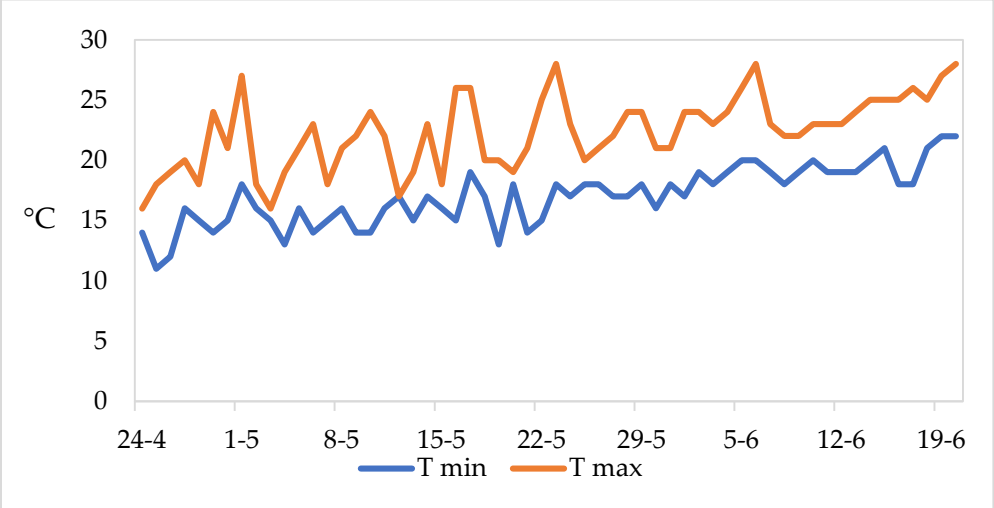

Figure 1. Daily maximum and minimum air temperatures under the high shaded greenhouse during plant growth.

During the trial, stomatal conductance was measured (38 and 50 days after sowing for *T. patula* and *A. houstonianum* respectively) on two young fully expanded, randomly chosen unshaded leaves of each plant with a diffusion leaf porometer (AP4, Delta-T Devices Ltd., Cambridge, England). On the same day, leaf color was determined with a colorimeter (Chroma Meter CR-400C, Minolta corporation, Ltd., Osaka, Japan) on the upper side of two leaves randomly chosen for each plant, and the parameters L* (brightness), a* (blue/yellow) and b* (red/green) (CIELab) were recorded. Flower color was also determined on fully bloomed flowers (one flower per plant for French marigold and three flowers per plant for flossflower). Chroma (C*) and hue angle (Hue°) were then calculated as $C^* = (a^{*2} + b^{*2})1/2$, Hue° = 180° + arctan(b*/a*) for II quadrant (−a*, +b* for all the leaves and for *Tagetes* flowers) and Hue° = 360° + arctan(b*/a*) for IV quadrant (+a*, −b* for *Ageratum* flowers) [31,32]. Total color difference (ΔE) was also calculated as $\Delta E = [(L^* - L^*_0) + (a^* - a^*_0) + (b^* - b^*_0)]1/2$, where L^*_0, a^*_0, and b^*_0 are the values from plants grown in the conventional pot cultivation system (no inoculum and 100% mineral fertilization).

Growth measurements were carried out when the plants reached sufficient development to be sold as bedding plants: the first flower open in the case of French marigold (46 after sowing) or the first inflorescence in the case of flossflower (58 days after sowing). The following parameters were measured: plant height, number of shoots and leaves per plant, stem diameter (1 cm below the first node), flower diameter for *Tagetes* and flower number for *Ageratum*. Stem diameter was measured with a digital caliper (Kompernass Parkside, Bochum, Germany). Plants were then destructively sampled: the leaves, the flower and the stem were separated and the roots were gently washed to eliminate the substrate and then each part was weighted. Then, they were dried in an oven at 85 °C until a constant weight was reached and weighed again to calculate the dry biomass accumulation. Before drying, the leaf area was measured on digital images of the leaves with 300 dpi resolution obtained with a flat scanner (Epson photo 4180, Seiko Epson Corporation, Suwa, Japan) and processed with ImageJ software (ver. 1.52a, National Institutes of Health, Bethesda, MD, USA).

Plant water use (PWU) and plant water use efficiency (WUE) were calculated from the amount of water consumed by each plant for each irrigation and fertigation event by weighing each pot with a digital scale before refilling the reservoir and after drainage of the excess water. Soon after plant thinning, the top of the pot was covered with a hard polyethene disk with a central hole for the plant stem to prevent evaporation from the substrate, and thus the amount of water evaporated during the trial was negligible. PWU was calculated as PWU (g FW L^{-1} H_2O) = plant fresh weight (g)/H_2O (L), and WUE was calculated as WUE (g DW L^{-1} H_2O) = plant dry weight (g)/H_2O (L).

Microbial responsiveness (MR) to the microbial biostimulant of the shoot or the roots was calculated as follows: shoot MR% = ((shoot dry weight inoculated/shoot dry weight non-inoculated) × 100); root MR% = ((root dry weight inoculated/root dry weight non-inoculated) × 100) [33].

The study was carried out using a completely randomized design. To determine the effect of the microbial biostimulant inoculum and fertilization on French marigold and flossflower, a two-way ANOVA was carried out. The homogeneity of error variances was tested by applying Bartlett's test. Normality of data was checked according to Kolmogorov–Smirnov test. To distinguish the differences among treatments and the interactions between factors, the mean values of each parameter evaluated were differentiated by the least significant differences (LSD) test at $p \leq 0.05$. Percentages were subjected to angular transformation prior to performing statistical analysis ($\Phi = \arcsin(p/100)^{1/2}$).

3. Results

3.1. Tagetes patula

At the end of the growing cycle, the plants inoculated with the microbial biostimulant were 1.1 cm taller than the others on average. The lowest plant height was measured in

the control plants (10.07 cm). The use of a full-strength mineral NS (100% MF) significantly increased plant height (11.30 cm), but the tallest plants were those fertigated with 100% OF (12.8 cm) or 50% OF (12.4 cm) (Table 1).

Table 1. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on plant characteristics of *Tagetes patula* plants.

Source of Variance	Plant Height (cm)	Stem Diameter (mm)	Shoot Number (n plant^{-1})
Microbial inoculum			
M+	12.1a	4.1a	9.4a
M−	11.0b	3.9b	8.4b
Fertigation			
Control	10.1d	3.7d	6.9b
100% MF	11.3c	4.3a	9.3a
50% MF	11.0cd	4.1b	8.3ab
50% MF + 50% OF	11.6bc	4.3a	10.2a
50% OF	12.4ab	3.8dc	8.9a
100% OF	12.8a	4.0bc	9.6a
Significance			
Microbial inoculum (M)	**	***	*
Fertigation (F)	**	**	***
M × F	ns	ns	ns

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; ; ** significant at $p \leq 0.01$; *** significant at $p < 0.001$.

The diameter of the stem was greater in the inoculated plants and was also significantly affected by fertigation (Table 1). The stem was thinnest in the control plants (3.7 mm on average for inoculated and uninoculated plants). A significant increase was found when supplementing the plants with 50% MF or 100% OF (4.0 mm on average), but the thickest stems were those of the plants fertilized with 100% MF or 50% MF + 50% OF (4.3 mm on average).

Even the number of shoots was greater in the plants inoculated with the microbial biostimulant. A significantly lower number of shoots per plant was recorded in the unfertigated control plants (6.9) compared to the fertigated plants (Table 1).

The microbial inoculum did not significantly influence the total fresh weight of the plant, but had a positive effect on stem and flower fresh weight (Table 2), the latter especially when the plants were fertigated with 50% MF + 50% OF (Figure 2). The plants supplied only with water had the lowest fresh biomass (19.4 g FW plant^{-1}) together with 50% OF (21.4 g FW plant^{-1}). The use of 100% OF NS significantly increased the plant fresh weight and similar values were found also using 50% MF and 50% MF + 50% OF. The highest value was observed with 100% MF (+42.2% than the control) (Table 2). A similar effect of fertigation treatments was observed for the fresh weight of the stem, leaves and roots that linearly increased as increasing NS concentration of both OF and MF, but with a greater increase with MF.

The inoculum did not influence the shoot/root fresh weight ratio, whereas a significant increase of this parameter was found using the NS-containing mineral fertilizers (+31.2% than control on average for 100% MF, 50% MF and 50% MF + 50% OF) (Table 2).

The microbial biostimulant did not influence the total dry weight of the plants (2.3 g on average), but significantly increased the dry biomass accumulation in the stem and in the leaves (+10.8% and +11.8, respectively). The fertigation treatments had a marked effect. When MF was used (100% MF or 50% MF, alone or integrated with 50% OF), the dry

weight of the plants increased up to 37% on average compared to the control. A lower effect was determined using only OF that increased total dry biomass by 15.4% and 20.7% compared to the control with 50% OF and 100% OF, respectively.

Table 2. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the fresh and dry biomass of *Tagetes patula* plants.

Source of Variance	Fresh Weight (g plant^{-1})						Dry Weight (g plant^{-1})						Dry Matter (%)	
	Plant	Stem	Leaves	Flower	Roots	Shoot/ Root	Plant	Stem	Leaves	Flower	Roots	Shoot/ Root	Shoot	Root
Microbial inoculum														
M+	24.66	2.69a	6.84	1.01a	14.12	0.74	2.34	0.41a	0.85a	0.14	1.01	1.39	13.3a	7.2
M−	22.81	2.53b	6.64	0.80b	12.82	0.77	2.26	0.37b	0.76b	0.15	0.95	1.35	12.8b	7.4
Fertigation														
Control	19.43d	1.88e	4.88e	0.79b	11.89c	0.63b	1.88d	0.30c	0.63d	0.13b	0.86b	1.23b	14.0a	7.2
100% MF	27.62a	3.15a	8.72a	0.88ab	14.87a	0.86a	2.58a	0.46a	0.99a	0.15ab	1.02ab	1.57a	12.6b	6.9
50% MF	24.56b	2.79bc	7.18b	0.93ab	13.66b	0.81a	2.41ab	0.41ab	0.85b	0.15ab	1.00ab	1.41ab	12.9b	7.3
50% MF + 50% OF	25.87b	2.88b	7.60b	1.07a	14.32ab	0.81a	2.48ab	0.41ab	0.86b	0.17a	1.06a	1.36ab	12.5b	7.4
50% OF	21.39cd	2.38d	5.67d	0.85ab	12.49bc	0.72ab	2.17c	0.37b	0.73c	0.13b	0.95ab	1.29ab	13.8a	7.6
100% OF	23.58bc	2.58cd	6.40c	0.96ab	13.64b	0.74ab	2.27bc	0.37b	0.78bc	0.14ab	0.99ab	1.30ab	13.0b	7.3
Significance														
Microbial inocul. (M)	ns	*	ns	*	ns	ns	ns	***	***	ns	ns	ns	***	ns
Fertigation (F)	***	***	***	*	*	**	***	***	***	**	**	**	***	ns
M × F	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; *** significant at $p < 0.001$.

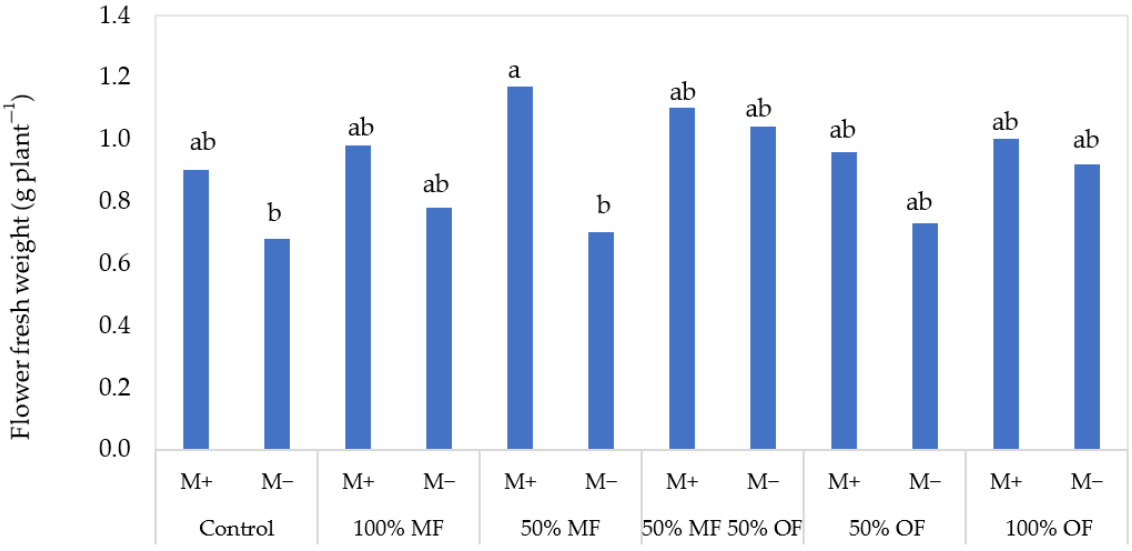

Figure 2. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the flower fresh weight of *Tagetes patula* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

A similar trend was found for the dry biomass of the leaves and the stem, even though in the latter, no significant difference was observed between the use of OF (both 50% and 100%) and the fertigation treatments containing 50% MF. The dry weight of the flower and the roots was less influenced by the fertigation treatments, with marked differences only between 50% MF + 50% OF and the control. The changes in the dry biomass of the different plant parts determined a significant change in the dry biomass partitioning only using 100% MF as shown by the shoot/root dry weight ratio (Table 2).

The epigeal dry matter percentage was higher in the plants inoculated with the microbial biostimulant (13.3%) and in those with limited nutrient availability (14.4% in the unfertilized control and 13.8% in the plants fertigated with 50% OF). No significant difference was determined by the experimental factors on the percentage of root dry matter.

Stomatal conductance was differently affected by the microbial biostimulant as a function of fertigation (Figure 3).

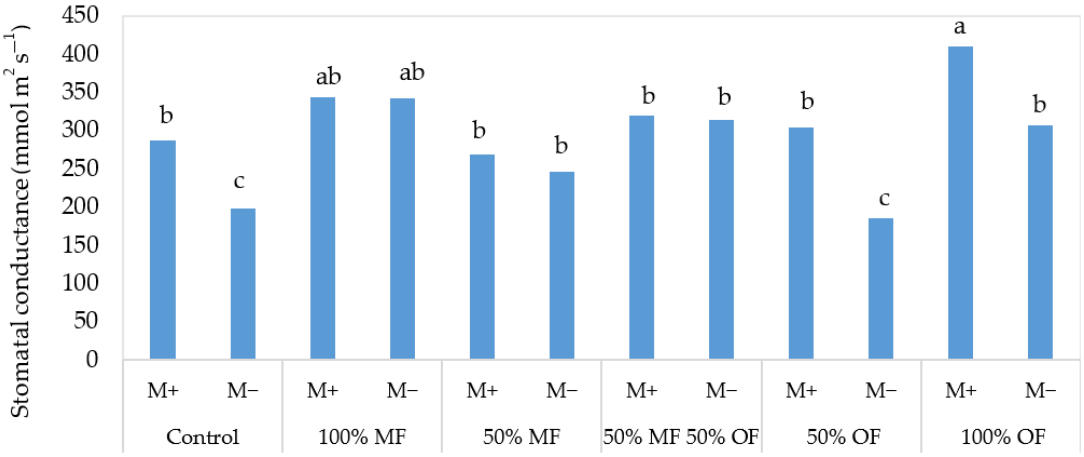

Figure 3. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the stomatal conductance of *Tagetes patula* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

It significantly increased in the inoculated plants grown without fertigation (control) and in the plants fertigated only with organic fluid fertilizer, whereas no variation was found between inoculated and uninoculated plants with the other NS tested. The highest stomatal conductance was recorded in the inoculated plants fertigated with 100% OF (410.0 mmol m^2 s^{-1}), while the lowest was found in the uninoculated plants fertigated with the control NS or 50% OF (191.4 mmol m^2 s^{-1} on average).

The fresh biomass produced per liter of irrigation water (PWU) was significantly higher in the plants inoculated with the microbial biostimulant (+8%). The lowest PWU was recorded in the control plants and in the plants fertigated with 50% OF (23.7 g FW L^{-1} H_2O) on average. The highest PWU was calculated in the plants fertigated with 100% MF (30.5 g FW L^{-1} H_2O), which did not statistically differ from 50% MF and 100% OF. The microbial inoculum slightly but significantly also influenced the water use efficiency. Similarly to PWU, a lower WUE was found in the control and 50% OF plants (2.2 g DW L^{-1} H_2O on average), whereas all the other fertigation treatments showed similar values (2.7 g DW L^{-1} H_2O on average) (Table 3).

Table 3. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on plant water use (PWU) and water use efficiency (WUE) of *Tagetes patula* plants.

Source of Variance	PWU (g FW L^{-1} H_2O)		WUE (g DW L^{-1} H_2O)	
Microbial inoculum				
M+	27.8	a	2.6	a
M−	25.6	b	2.5	b
Fertigation				
Control	23.4	c	2.1	b
100% MF	30.5	a	2.9	a

50% MF	27.7	ab	2.7	a
50% MF 50% OF	27.1	b	2.7	a
50% OF	23.9	c	2.3	b
100% OF	27.7	ab	2.7	a
Significance				
Microbial inoculum (M)	*		*	
Fertigation (F)	***		***	
M × F	ns		ns	

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; *** significant at $p < 0.001$.

The responsiveness of the plants to the microbial inoculum varied differently for the shoot and the root according to the fertigation treatments. The microbial responsiveness (MR) of the roots was 118.7% on average for the control plants, 50% and 100% MF and significantly decreased in all the plants fertigated with NS containing OF (106.9%) (Figure 4). The MR of the shoot was lower in the control plants and in the plants fertigated with 100% MF and 50% MF + 50% OF (104.6% on average) and increased when lowering the mineral nutrient content in the NS (50% MF) or when using OF (114.2% on average).

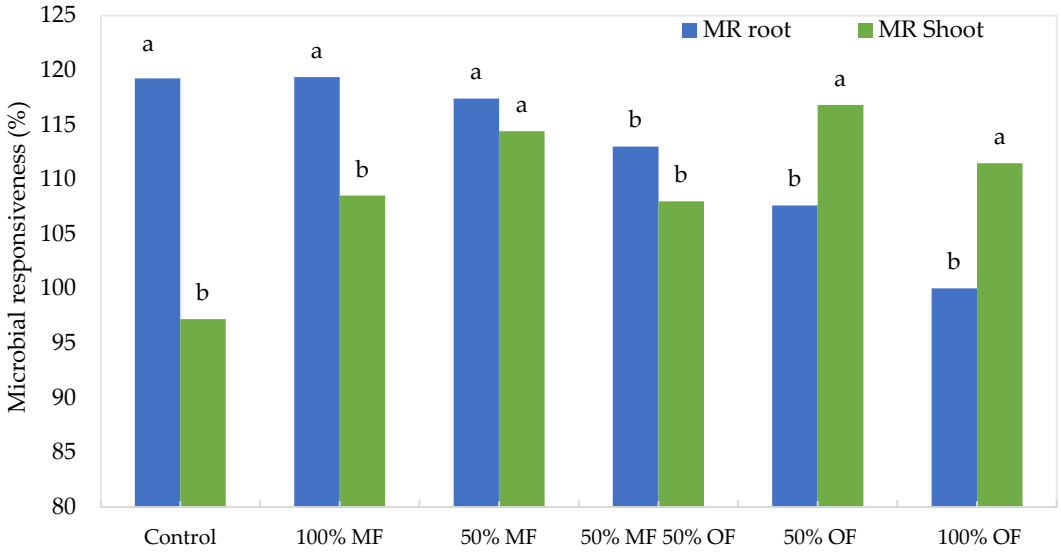

Figure 4. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the shoot and root microbial responsiveness (MR) of *Tagetes patula* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

The number of leaves was influenced only by fertigation. The lowest leaf number per plant was found in the control plants and in 50% OF plants (48.1). The use of 100% OF increased the leaf number by 22.2%, but the leafiest plants were those fertigated with 100% MF (+43.6% than control). The other fertigation treatments showed intermediate values (Table 3).

The leaf area of the plants was wider in the plants inoculated with the microbial biostimulant. Fertigation had a positive effect on plant leaf area with a significant increase compared to the control (258.3 cm^2 $plant^{-1}$) when using the highest dose of OF (+23.0% for 100% OF compared to control. Leaf area increased even more when using nutrient solutions with 50% MF (+48.7% on average for 50% MF and 50% MF + 50% OF) and up to 486.6 cm^2 $plant^{-1}$ (+81.4%) with 100% MF (Table 4).

Table 4. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the characteristics of leaves and flowers of *Tagetes patula* plants.

Source of Variance	Leaf Number	Leaf Area (cm² plant⁻¹)	Leaf Color				Flower Diameter (mm)	Flower Color			
			L*	Chroma	Hue	ΔE		L*	Chroma	Hue	ΔE
Microbial inoculum											
M+	59.5	367.9a	45.0	26.3a	124.5	3.8	30.6	83.2	94.0	97.4	6.7a
M−	57.3	344.1b	43.9	24.7b	125.5	3.1	29.6	81.5	92.7	96.7	6.0b
Fertigation											
Control	47.8c	258.3d	45.8	26.1ab	123.8	5.5a	29.0	81.8	99.6a	96.2	4.9b
100% MF	68.6a	468.6a	43.1	22.1c	123.8	1.8d	30.1	80.9	97.8a	95.9	3.6c
50% MF	61.9ab	383.4b	44.5	25.8ab	126.4	3.3c	30.2	84.3	91.8b	98.6	6.4bc
50% MF 50% OF	65.4ab	384.8b	44.6	26.8ab	126.7	3.1c	31.4	82.8	97.4a	96.9	4.6c
50% OF	48.3c	293.0cd	45.4	28.2a	125.0	4.4b	30.0	80.9	89.6b	96.3	7.3b
100% OF	58.4b	317.8c	43.3	24.1bc	124.2	2.9c	29.9	83.4	84.1c	98.4	11.5a
Significance											
Microbial inoculum (M)	ns	***	ns	*	ns	ns	ns	ns	ns	ns	**
Fertigation (F)	***	***	ns	**	ns	**	ns	ns	*	ns	**
M × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; *** significant at $p < 0.001$.

Leaf color is a very important characteristic of ornamental plants. No significant difference was determined by the experimental factors on L* and hue values, while the vividness of the color (chroma) significantly changed as a function of the microbial inoculum and fertigation. The color of the leaves was more saturated in the plants inoculated with the microbial biostimulant. On the contrary, the chroma decreased when increasing the amount of organic nutrients (100% OF) or even more with mineral nutrients (100% MF). The color difference (ΔE) increased as reducing mineral or organic supply, was visible with 50% OF, and was greatest in the control plants (Table 3).

The treatments did not affect flower diameter, which was 30.1 mm on average (Table 3).

The color of the flowers was affected only by fertigation, and as leaf color, it affected only the chroma, which was 99.6 in the control plants and decreased with 50% MF or OF and reached the lowest value with 100% OF (84.1) (Table 3). The difference among flower colors increased by reducing the amount of nutrient supplied to the plants, but the highest difference was recorded with the highest amount of organic nutrients (100% OF).

3.2. Ageratum houstonianum

The height of the flossflower plants was influenced only by the fertigation treatments, with the highest value recorded with 50% OF (19.8 cm) and the lowest with 100% MF (16.6 cm) (Table 5).

T**able 5.** Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on plant characteristics of *Ageratum houstonianum* plants.

Source of Variance	Plant Height (cm)	Stem Diameter (mm)	Shoot Number (n. plant⁻¹)
Microbial inoculum			
M+	19.5	5.8a	4.7
M−	17.3	5.2b	4.6
Fertigation			
Control	17.8ab	5.1c	3.3

100% MF	16.6b	5.8a	5.9
50% MF	17.9ab	5.6ab	4.3
50% MF 50% OF	19.3ab	5.8a	4.9
50% OF	19.8a	5.3bc	3.9
100% OF	18.9ab	5.5ac	4.0
Significance			
Microbial inoculum (M)	ns	***	*
Fertigation (F)	*	**	***
M × F	ns	ns	*

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; *** significant at $p < 0.001$.

The diameter of the stem significantly increased by 10% in the plants inoculated with the microbial biostimulant compared to the uninoculated plants. Without fertigation (control plant only watered), the stem diameter measured 5.1 mm. A significantly thicker stem was recorded in the plants fertigated with NS containing MF, especially those with the highest amount of nutrients (5.8 mm on average for 100% MF and 50% MF + 50% OF) (Table 5).

The tendency to branch was highest in the plants fertigated with 100% MF (5.9 branches on average), followed by those inoculated and fertigated with 50% MF + 50% OF (5.3 branches) (Figure 5). The microbial inoculum determined an increase in the shoot number compared to the uninoculated plants by about 30% in the control plants and in the plants fertigated with 50% MF and by about 18% in the plants fertigated with 50% MF + 50% OF and 100% OF.

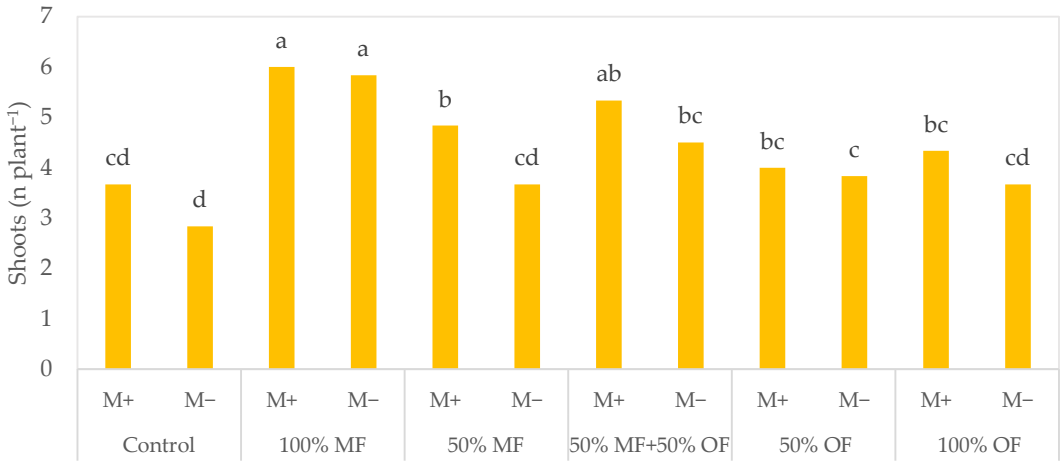

Figure 5. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the shoot number of *Ageratum houstonianum* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

The total fresh weight of the plants was positively influenced by the microbial biostimulant, and this was mainly due to the increase in the fresh weight of the stems and leaves, as the fresh weight of flowers and roots was not affected by the microbial inoculum. Thus, the shoot/root fresh weight ratio increased in the inoculated plants (Table 6).

The fertigation determined an increasing trend of the plant fresh weight as increasing nutrient concentration, ranging from 21.2 g FW plant^{-1} in the control plants up to 28.8 g FW plant^{-1} (+35.8%) with only OF or up to 29.8 FW plant^{-1} (+40.3%) with only MF, but the greatest fresh biomass was recorded with 50% MF + 50% OF (+49.5%). All the fertigation treatments increased the stem fresh weight, even if to a lower extent for 50% OF (+20.6% with

50% OF and +44.5% on average with the other fertigation treatments). The fresh weight of the leaves of control plants was 7.0 g FW plant^{-1} and increased significantly with 100% OF, but a higher fresh biomass accumulation was recorded in the plants fertigated with NS containing 50% MF (+46.7% on average) or with 100% MF (+70.0%). The flower fresh weight raised significantly only in the plants fertigated with 50% MF. The root biomass increased only by increasing the amount of OF and was highest using 50% MF + 50% OF (+50.9%). This increase was similar to those recorded in the epigeal parts of the plant, so there was no significant variation in the shoot/root fresh weight compared to the control in the plants supplemented with NS containing OF, whereas this parameter increased in the NS with only MF up to +38.0% (100% MF).

Table 6. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the fresh and dry biomass of *Ageratum houstonianum* plants.

Source of Variance	Fresh Weight (g plant^{-1})						Dry Weight (g plant^{-1})						Dry Matter (%)	
	Plant	Stem	Leaves	Flower	Roots	Shoot/ Root	Plant	Stem	Leaves	Flower	Roots	Shoot/ Root	Shoot	Root
Microbial inoculum														
M+	28.38a	5.62a	10.11a	1.90	11.71	1.52	3.10	0.90	1.07	0.23	1.01	2.18	12.5	8.6
M−	26.45b	4.82b	8.71b	1.44	10.69	1.40b	2.89b	0.81b	0.96b	0.17	0.86	2.25	13.0	8.0
Fertigation														
Control	21.20d	3.92c	7.01d	1.32b	8.95 d	1.37c	2.42d	0.65c	0.84b	0.16	0.80c	2.06ab	13.4a	8.9ab
100% MF	29.75ab	5.72a	11.92a	1.84ab	10.28 cd	1.89a	3.18ab	0.92ab	1.22a	0.22	0.84bc	2.80a	12.1c	8.2b
50% MF	28.20b	5.44a	10.10b	1.92a	10.74 bd	1.63b	3.22ab	0.91ab	1.10a	0.24	0.97ab	2.33ab	12.9ac	9.0a
50% MF 50% OF	31.69a	5.93a	10.47b	1.78ab	13.51 a	1.37c	3.38a	0.98a	1.14a	0.21	1.07a	2.17ab	12.8ac	7.9b
50% OF	25.39c	4.73b	7.83d	1.46ab	11.38 bc	1.23c	2.76c	0.80b	0.84b	0.18	0.94ab	1.93b	13.0ab	8.3ab
100% OF	28.80b	5.57a	9.13c	1.72ab	12.39 ab	1.32c	3.02bc	0.88ab	0.95b	0.21	0.98ab	2.08ab	12.4bc	7.9b
Significance														
Microbial inoculum (M)	**	***	***	ns	ns	***	*	*	***	ns	ns	ns	ns	ns
Fertigation (F)	***	***	***	*	***	***	***	***	***	ns	**	**	**	**
M × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; *** significant at $p < 0.001$.

Unlike what was observed in the French marigold plants, the microbial biostimulant positively influenced the total dry weight of the flossflower plants (Table 6). The increase in the total dry biomass determined by the microbial biostimulant could be ascribed to the increase in stem (+10.8%) and leaf (+12.1%) dry biomass.

As found for the fresh biomass, even the dry biomass of the flossflower plants was significantly increased by the fertigation, especially the NS containing MF, regardless of the percentage of nutrients (up to +39.8% with 50% MF + 50% OF compared to control). The dry weight of the stems of the control plants was 0.65 g DW plant^{-1} and increased significantly to 0.80 g DW plant^{-1} with 50% OF and up to 0.98 g DW plant^{-1} with 50% MF + 50% OF. As regards the leaves, the dry weight increased only when using MF alone or in combination with OF (+37.0% on average). A different trend was observed as regards the root dry weight, as it increased significantly with 50% MF + 50% OF (+33.8% compared to control) followed by all the other fertigation treatments, excluding 100% MF. Because of dry biomass changes in the different plant parts, the shoot/root ratio showed differences due to microbial inoculum only in the control plants and in the plants with 100% MF that had the highest shoot/root dry weight ratio when inoculated with the microbial biostimulant (Figure 6).

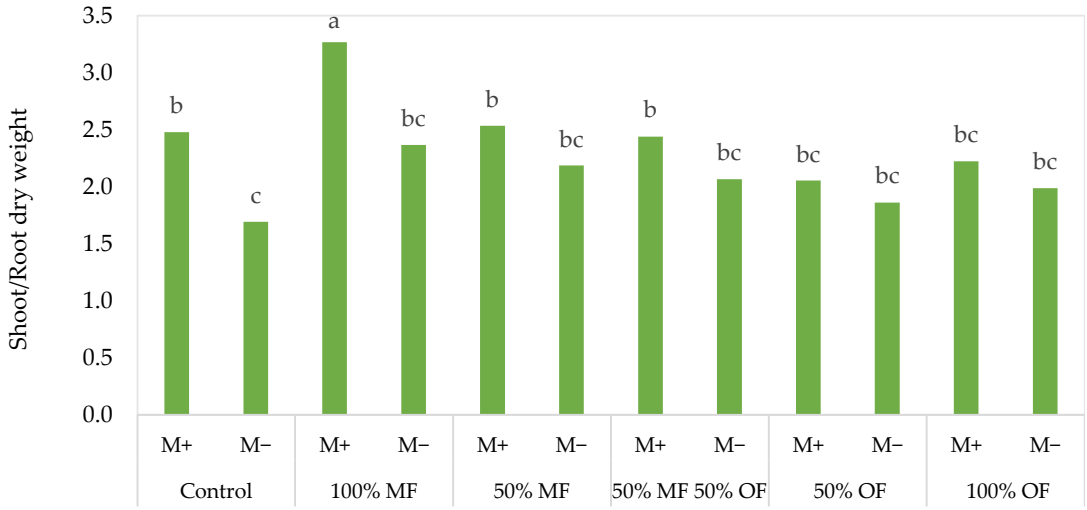

Figure 6. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the shoot/root dry weight of *Ageratum houstonianum* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

The percentage of epigeal and root dry matter was affected only by the fertigation, with differences not always stark (Table 6). The epigeal dry matter percentage was highest in the control plants and tended to decrease in al the fertigated treatments, with significant drops with 100% MF and 100% OF. The root dry matter percentage of the fertigated plants did not change significantly compared to the control plants (8.9%) and ranged from 8.0% on average with 100% MF, 50% MF + 50% OF and 100% OF to 9.0% with 50% MF.

The microbial inoculum determined a significant increase in stomatal conductance in the control plants, and the lowest stomatal conductance was recorded in the uninoculated plants (214.5 mmol m^2 s^{-1}). The plants fertigated with 100% MF had the highest stomatal conductance when inoculated with the microbial biostimulant (Figure 7).

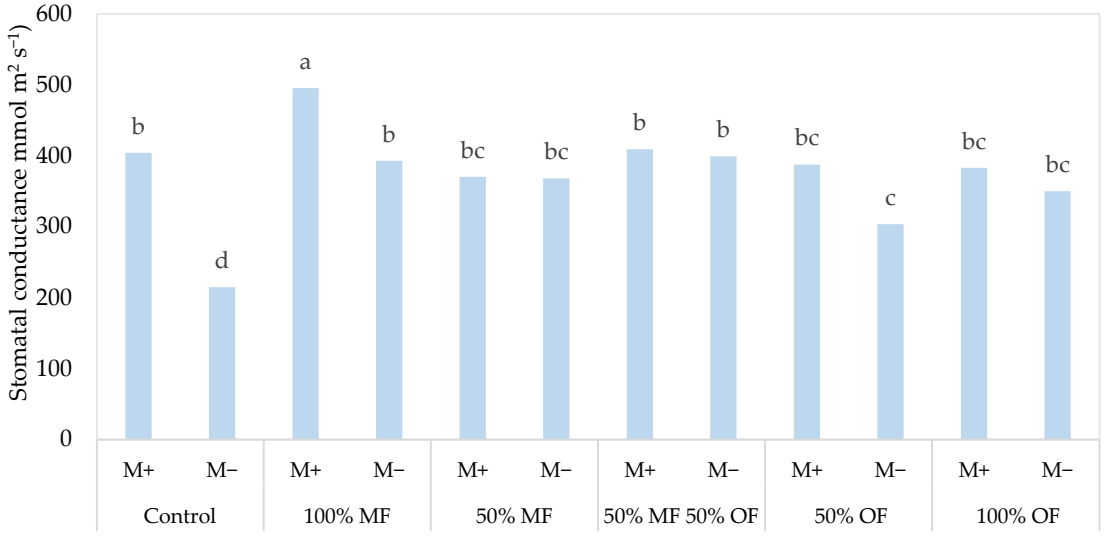

Figure 7. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the stomatal conductance of *Ageratum houstonianum* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

The production of fresh and dry matter per unit of water consumed (PWU and WUE) was not influenced by the microbial biostimulant, but showed variations due to the fertigation treatments. The use of mineral nutrients in the NS increased both PWU and WUE to the maximum level. An increase in these parameters was also recorded when increasing OF concentration, but to a lower extent compared with MF (Table 7).

Table 7. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on plant water use (PWU) and water use efficiency (WUE) of *Ageratum houstonianum* plants.

Source of Variance	PWU (g FW L^{-1} H_2O)	WUE (g DW L^{-1} H_2O)
Microbial inoculum		
M+	31.4	3.4
M−	30.8	3.4
Fertigation		
Control	26.5c	3.1d
100% MF	33.8a	3.6a
50% MF	31.7ab	3.6a
50% MF 50% OF	33.2a	3.6ab
50% OF	29.7b	3.2cd
100% OF	31.9ab	3.3bc
Significance		
Microbial inoculum (M)	ns	ns
Fertigation (F)	***	***
M × F	ns	ns

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; *** significant at $p < 0.001$.

The microbial responsiveness of shoot and root was peculiar: it was little influenced in the case of shoot growth, while it was higher for the roots when using the full dose of mineral fertilizers (100% MF) and in the control (Figure 8).

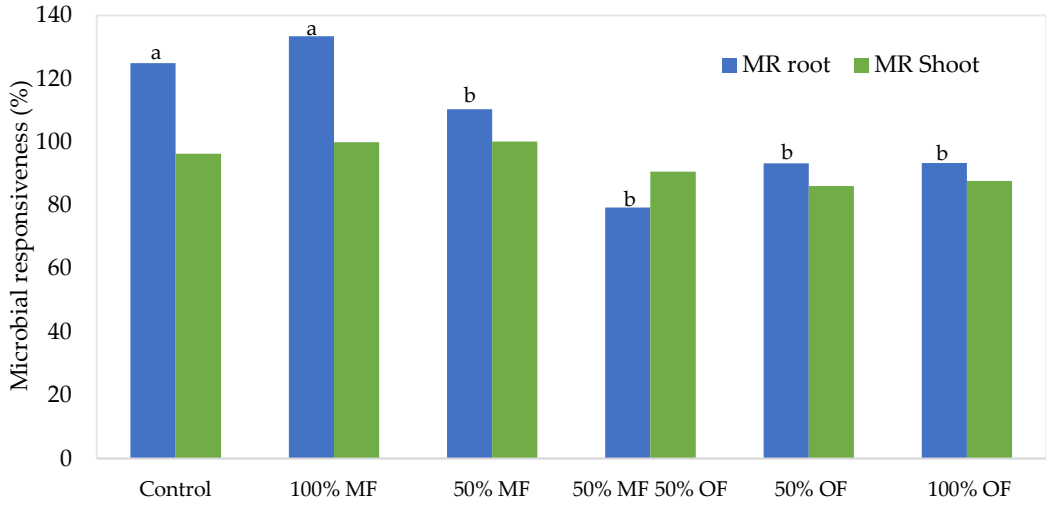

Figure 8. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the shoot and root microbial responsiveness (MR) of *Ageratum houstonianum* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

The plants of *A. houstonianum* inoculated with the microbial biostimulant produced 11.5% more leaves than the uninoculated plants (Table 8). The plants not fertigated had 27.8 leaves plant^{-1}. The leaf number increased up to 34.7, on average, fertigating the plants with 50% MF or OF; a further increase was found with 100% OF or with 50% MF + 50% OF, while the leafiest plants were those fertigated with 100% MF (43.1 leaves plant^{-1}).

As found for the leaf number, the total leaf area increased in the plants inoculated with the microbial biostimulant (+13.0%). The fertigation treatments greatly affected the plant leaf area, which was 275.9 cm^2 plant^{-1} in the control plants. A linear increasing trend was found when increasing the amount of nutrient, but OF increased the leaf area to +27.7%, whereas MF increased the leaf area by 42.1% with 50% MF and by 78.3% with 100% MF. A good effect was also obtained with 50% MF + 50% OF that increased the leaf area by 56.8% (Table 8).

Table 8. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the characteristics of leaves and flowers of *Ageratum houstonianum* plants.

Source of Variance	Leaves	Leaf Area	Leaf Color				Flower	Flower Color			
	(n. plant^{-1})	(cm^2 plant^{-1})	L*	Chroma	Hue	ΔE	(n. plant^{-1})	L*	Chroma	Hue	ΔE
Microbial inoculum											
M+	38.4a	399.1a	42.7	28.5	125.9	2.6	33.4	37.4ns	36.8a	324.7b	18.2a
M−	34.5b	353.2b	43.3	28.4	126.3	2.6	27.9	38.1	23.7b	332.9a	8.8b
Fertigation											
Control	27.8d	275.9f	42.5	28.4	126.2	3.9a	27.2b	41.8a	28.2b	331.5a	19.3a
100% MF	43.1a	491.9a	41.1	26.5	127.4	1.2c	30.3ab	38.8a	33.4ab	325.1b	14.0b
50% MF	35.5c	392.0c	43.4	28.6	126.1	2.3b	33.3a	38.1a	36.6a	325.2b	17.6a
50% MF 50% OF	39.3b	432.7b	42.8	28.8	126.0	1.2c	31.5ab	37.2ab	37.7a	330.6a	7.5bc
50% OF	33.8c	312.1e	44.1	29.7	125.2	3.4a	27.3b	35.6b	26.8b	323.9b	18.3a
100% OF	39.2b	352.2d	44.1	28.7	125.7	3.4a	34.7a	35.1b	18.8c	336.6a	4.3c
Significance											
Microbial inoculum (M)	**	*	ns	ns	ns	ns	ns	ns	*	*	*
Fertigation (F)	***	**	ns	ns	ns	*	**	*	*	*	**
M × F	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	ns

Data within a column followed by the same letters do not differ significantly according to the LSD test. Significance: ns = not significant; * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; *** significant at $p < 0.001$.

The experimental factors did not significantly affect the leaf color components (L*, chroma and hue). Nevertheless, significant leaf color differences (ΔE) were found in the control (3.91) and with organic fertigation (3.40 on average), while ΔE was lower and less perceptible with 100% MF, and with 50% MF + 50% OF (1.2 on average).

The number of flowers was differently affected by microbial inoculum in the different fertigation treatments. The lowest number of flowers was recorded in the uninoculated plants of the control and with 50% MF + 50% OF and both inoculated and uninoculated plants with 50% OF. The microbial inoculum significantly increased the flower number in all the treatments with MF and in the control. The highest number of flowers was then found in the inoculated plants fertigated with 100% MF, 50% MF, 50% MF + 50% OF and 100% OF (Figure 9). As regards the colorimetric parameters of the flowers, the microbial biostimulant made the color more saturated, with a reduction in hue angle ending in a higher color difference (ΔE = 18.2). The different fertigation treatments influenced the colorimetric parameters: the color of the flowers was darker when supplementing OF; the saturation (chroma) increased using MF in the NS and decreased with 100% OF; and the hue angle decreased using 50% or 100% MF and with 50% OF. These color differences were greater in the control flowers and 50% MF and 50% OF and lower in 100% OF.

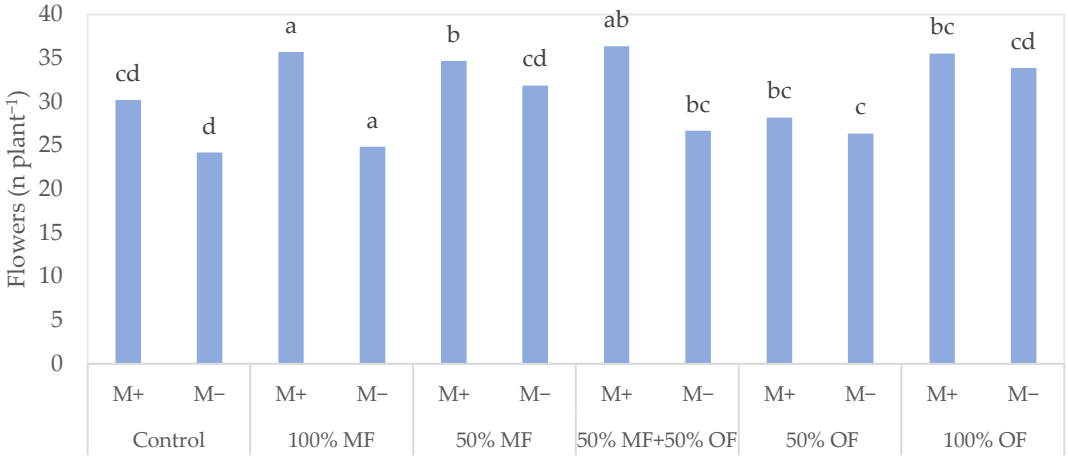

Figure 9. Effect of microbial biostimulant inoculation (M− and M+: uninoculated and inoculated plants) and mineral (MF) and/or organic (OF) fertigation on the number of flowers of *Ageratum houstonianum* plants (bars with different letters are significantly different at $p < 0.05$ according to the LSD test).

4. Discussion

The seeds of *T. patula* and *A. houstonianum* germinate from 10 to 30 °C without relevant variations. For many non-dormant bedding plants, the optimum growth temperature is between 24 and 30 °C [34]. According to Pramuk and Runkle [35], bedding plants develop quickly when grown warm (approximately 27° C) and under high light, and days to flowering decrease as temperature increases. In our trial, a cooler temperature, below 24–27 °C, was not detrimental to plant quality. Generally, poor plant quality occurs when plants are grown warm with high light levels. Plants are of the best quality when grown at cooler temperatures with high light levels, such as the climatic condition we had during our research.

One of the problems of the floriculture industry is the environmental impact of leachates containing high concentrations of chemical fertilizers. Extremely high concentrations of nitrate-N have been found under commercial greenhouses in the USA [36]. So many sellers, buyers, and researchers promote the use of organic fertilizer in floriculture for pot cultivation to reduce pollution and have more environmentally friendly products. The results of the studies on organic versus mineral fertilization do not always match. Some authors have found few differences in the macronutrient concentration in the edible part of the crops in a comparative study of organic vs. mineral fertilization [37], others showed that organic fertilization in a soilless system may adequately cover the needs of leafy plants for nutrients [38,39], while still others reported that the conventionally fertilized leafy plants are more productive than the plants grown with organic fertilizer and the yield tends to be greater with conventional fertilizers compared to organic fertilizers [40]. The results are never unequivocal and depend on crop species and varieties, nutrient type, climatic conditions, fertilization timing, handling and storage after harvesting and year of the study [37,41,42].

In our study, different levels of organic and inorganic fertilizer determined few differences in growth. The six treatments (control, fully mineral and fully organic fertigation, 50% mineral and organic fertigation, alone and in combination) determined little differences in the growth of bedding plants of the tested species. The plants of French marigold and flossflower had a proper height, good stem diameter and number of leaves even when only partially supplied with organic fertilizers. Only a few attributes of bedding plant quality (e.g., leaf area and Chroma) were negatively affected by decreasing conventional fertilization and increasing organic fertilization. Usually, the differences in the growth rate

can be ascribed to a difference in the availability of the nutrients, especially in the case of the N that derives from organic sources that become available over time [43]. Organic fertilizer requires consistent microorganism activity for decomposition and release of nutrients [40]. Unlike inorganic fertilizer, organic nutrients from plant and animal-based residues are often not immediately available to the plant and must be converted into plant-available forms [44] through a mineralization process in which substrate microbes metabolize organic carbon (C) and convert organic N compounds into ammonium and nitrate. The rate of microbe-mediated mineralization of the organic fertilizers is highly variable and depends on several factors. The timing of N availability is often a critical limiting factor of organic fertilizer use [45], and it is often not clearly predictable due to the numerous factors involved, such as the climatic conditions. In our research and during the cultivation period, the temperature (>15 °C and <35 °C) was ideal for nitrifiers bacteria [46] probably allowing sufficient mineralization rates to make the organic substances in the OF almost fully meet the needs of our bedding plants.

Proper growth and bedding plant quality are often related to an appropriate response to CO_2 [47], an increase in photosynthetic capacity, a higher stomatal conductance and an increase in water use efficiency [48]. Our findings on stomatal conductance, PWU and WUE confirmed that organic fertigation under good temperature and fast mineralization conditions does not cause a lack of ready and available nitrogen.

The effect of different biostimulants on stomatal conductance and water use efficiency (WUE) has been widely studied to assess their influence on plant water relations. Stomatal conductance rates are usually higher in the plants inoculated with mycorrhizal fungi plants than in the non-inoculated [49]. A lot of studies explained how the symbiosis influences host stomatal conductance: increased water uptake via the soil (extraradical) hyphae [50], altered hydraulic conductivity of the roots [51], altered hormonal relations [51], and altered root system architecture [52]. Moreover, plant growth-promoting rhizobacteria (PGPR) such as *Bacillus* spp. can determine an increase of stomatal conductance along with an improvement in photosynthetic rate [53–55], thus enhancing dry biomass accumulation and WUE. Our results on *T. patula* and *A. houstonianum* seem to confirm that mycorrhizal fungi and PGPR, as those contained in the microbial biostimulant tested, impact plant water relations and can increase stomatal conductance, as already found by Ibrahim et al. [56], who found that the stomatal conductance of *G. intraradices*-colonized sorghum plants were significantly greater than uncolonized plants. Regarding fertigation treatment and in agreement with other research [57,58], the unfertilized control showed the lowest value of stomatal conductance for both the bedding plants species. The 100% organic fertigation and the combined 50% organic and 50% inorganic fertigation resulted in higher increase in stomatal conductance, like 100% mineral fertigation. Several reports in the literature showed an increase in WUE due to various biostimulant based on seaweed extract [59,60], amino acids [61], rhizobacteria [62] and mycorrhizal fungi [49,63,64]. Data from our research confirmed that WUE improved using a consortium of microorganisms. Furthermore, some authors [65] stated that WUE increases with increased fertilizer rate. We also observed for both bedding plant species that by halving the mineral fertilizer in the nutrient solution and integrating it with organic fluid fertilizers, the WUE levels showed small variations.

Stomata regulate carbon assimilation rates and water loss, exerting a controlling influence on photosynthesis, hydration, and ultimately biomass accumulation [66]. As reported by several studies [67–69], even in our research, the microbial biostimulant had a positive effect on biomass accumulation, especially in the stem and leaves. Regarding the fertigation treatments, we did not observe a high dry matter accumulation using organic fertilizer, as reported by some authors [70]: the 100% MF and the 50% MF + 50% OF resulted in almost equivalent dry biomass for both species.

The arbuscular mycorrhizal fungi (AMF) present in commercial biostimulants are believed to be obligatory symbiotic, so no direct relationship is expected between them and organic matter as an energy source. Despite this, AMF responds markedly to the

presence of organic matter in their environment [71,72] as well as to organic fertilization [73–75]. For all the measured parameters, we always observed a trend, sometimes slight, sometimes marked, with better plant performance in the presence of the microbial biostimulant inoculum we used, containing mycorrhizal fungi, *Trichoderma*, and PGPR (*Agrobacterium radiobacter*, *Bacillus subtilis* and *Streptomyces)*. *Trichoderma* is well known for improving plant development, seed germination, chlorophyll content and yield, size and/or number of flowers and/or fruits [76–79]. The effects ascribed to the *Trichoderma* are the solubilization of phosphate and micronutrients [80], the release of secondary metabolites [81], or auxin modifications [82,83] that promote plant development. Synergistic effects on biocontrol have been found in many combinations of fungi and bacteria [84]. Research on fungi and bacteria suggests that the mixtures of microorganisms work complementarily in a biostimulant consortium [84] and determine a synergic interaction by providing nutrients, removing inhibitory products, and stimulating beneficial physiological traits [85]. Among the bacteria, *Agrobacterium radiobacter*, *Bacillus subtilis* and *Streptomyces* have in common many effects: they secrete plant growth promoting substances such as phytohormones [86–88], proline [89], vitamins, and antifungal metabolites, improve zinc and phosphate solubilization [90–92] and Fe mobilization [93] in vitro and in soil, even under abiotic stress conditions [18,94,95]. Organic fertilization effectively increases soil bacteria [96], while mineral fertilization reduces soil bacterial network complexity and connectivity [97]. In agreement with previous research [98] on *T. patula*, the microbial biostimulant used in our study improved the stem thickness (a positive characteristic of bedding plants during transport and marketing), the number of shoots (an essential parameter for decorative species), the fresh weight of flower and the dry weight of stem and leaves. Plant height also increased, but not so much as to become a problem for bedding potted plants that require a compact habitus. Microbial biostimulant increased growth at low and high nutrient levels [99].

The microbial biostimulant improved the stomatal conductance, the stem diameter and its fresh and dry weight, the number of shoots, the number and fresh and dry weight of the leaves of *A. houstonianum* plants. The bedding plants of this species grew better with the highest level of mineral fertigation, but comparable results were obtained by replacing half of the dose of the mineral fertilizers with the organic fluid fertilizer. Even if this fertigation treatment (50% MF + 50% OF) reduced the number of shoots and leaves and the total leaf area (−12% compared to 100% MF), it did not affect the flowering.

Leaf and flower color is one of the most essential visual attributes for a high-quality pot plant, as it can affect product attractiveness to buyers [100] and is usually the first aspect evaluated and associated with product quality [101]. Studies on the effects of organic fertilization or microbial biostimulant on the color of leaves and flowers are limited. Color measurements could help in evaluating the effect of fertilizers and biostimulants, as color variations can correspond to a specific plant response to the nutritional status or some stress conditions. For example, a deficiency in mineral nutrients may cause different color changes on plant leaves [102,103]. Leaf color is primarily due to its chlorophyll content, which can be affected by substrate fertility, especially as regards nitrogen content and availability. Similarly to what was found for the basil leaf in soilless cultivation [104], differences were recorded in the color of the leaves between the fertigation treatments. The flossflower plants partially fed with organic fertilizers (50% MF+ 50% OF) in the nutrient solutions did not show differences in color compared to the uninoculated 100% MF plants used as a reference but showed more marked differences from control plants with lower mineral N supply, thus indicating that the mix of the organic and mineral nutrient solution allowed plants to have enough assimilable nutrients [104]. The change in fertilizer source from fully mineral (100% MF) to fully organic (100% OF) determined remarkable changes in the color of the flowers, which tended to be less saturated and vivid and visibly different from the reference plants ($\Delta E > 3$) [105]. Changes in leaf and flower color were also due to the biostimulant inoculum: notable differences in color ($\Delta E > 18$) were caused by the microbial inoculum in *Ageratum* flowers, for example, and thus further studies

should be carried out to better investigate how the inoculated microorganisms can alter flower color parameters.

The responsiveness to the microbial inoculum of shoots and roots varied for the tested species, confirming that the compatibility between plant species and beneficial microorganisms can be different. The response of plant growth to the inoculum with beneficial microorganisms can vary a lot, as it is the result of multiple factors and depends on the environmental conditions and the genotypes of both the plant and the microbial species. Moreover, it is well known that nutrient supplementation affects AMF symbiosis and microorganism development in the soil, and sometimes nutrient addition can shift the symbiotic nature from mutualism to parasitism, ending in the loss of their beneficial effect. This shift depends also on the plant species, as found in our experiment, where the microbial responsiveness of French marigold and flossflower differed also as a function of the amount and nature of the fertilizers used for fertigating the plants. We observed that the microbial inoculum improved more the shoot than the root growth of *T. patula* plants with organic fertigation (50% OF and 100% OF), while the *A. houstonianum* plants seemed little influenced by the microbial inoculum as regards the shoot growth. For some species, there is evidence that microbial-inoculated plants invest more into shoot than in root growth [104]. Linderman et al. [33] reported that after microbial inoculation, large variation in the size of root systems was observed many times without variation in shoot biomass, as we observed for *Ageratum*. We also found that the plants of *T. patula* fertigated with a lower dose of MF or with OF had a greater increase in shoot growth when inoculated with the microbial biostimulant compared to 100% MF or control. Thus, both high nutrient availability (100% MF) and low nutrient availability (control) can limit the beneficial growth promotion effect of the microbial consortium, but this can vary depending on crop species, as found with the small variation in the responsiveness to the microbial inoculum related to the shoot biomass accumulation.

5. Conclusions

This research on *T. patula* and *A. houstonianum* showed that fertigating plants inoculated with a commercial microbial consortium with reduced rates of mineral fertilizer coupled with an organic fluid fertilizer stimulated plant growth similarly to what was observed with a conventional fertigation with mineral fertilizers. The mineral fertilizer could be reduced by 50% and integrated with an organic fluid fertilizer in the inoculated plants without affecting plant ornamental value characteristics. The results indicated the possibility of increasing the sustainability of producing pot plants of *T. patula* and *A. houstonianum* for the bedding plant market during springtime in the Mediterranean area. Further studies on microbial consortia and organic fertilizer sources could help in better understanding their mechanism of action and thus improving the quality parameters of ornamental pot-plant species.

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