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Effect of Microbial Biostimulants and Organic Fertigation on Nursery Production of Lettuce Transplants †

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Abstract: Organic liquid fertilizers are available for transplant fertigation but their efficacy and rates for use have not been evaluated. Moreover, plant growth-promoting rhizobacteria have been applied to different vegetable crops but there is still no information on the combined effect of microbial biostimulant application and organic fertigation on organic production of lettuce seedlings. This study aimed to assess the efficacy of two commercial microbial biostimulants (TNC Bactorr^{S13} and Flortis Micorrize) to enhance growth and quality of lettuce seedlings fertigated with a nutrient solution containing 0, 7, 14, and 28 mL L^{-1} of an organic liquid fertilizer (NK 3–4). The use of the microbial biostimulants modified seedling growth and its response to organic liquid fertilizer levels. They had a growth-promoting effect on the unfertilized seedlings and modified the response of lettuce seedlings to organic fertigation but to different extents for TNC Bactorr^{S13} and Flortis Micorrize.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article yet reasonably common within the subject discipline.)

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1. Introduction

Vegetable seedlings (transplants) are frequently used for establishing crops in open fields and under greenhouses [1]. Transplants are generally raised in trays filled with a limited volume of substrates with low nutrient content by vegetable nurseries that aim to produce high-quality transplants in a short time and use intensive cultivation systems with frequent supplementation of water and fertilizers. Incorrect seedling growth management can adversely affect seedling quality resulting in weaker transplants that underperform in the field [1]. The fertigation rate can influence the growth and quality characteristics of vegetable seedlings and may also increase growth rate, yield and quality of the crops [2,3].

The transplants used for establishing organic cultivations should be produced in accordance with the EU rules and principles on organic products [4]. Organic transplant must be produced by organic nurseries that use only seeds, substrates and fertilizers that have been authorized for use in organic production. The nutrients needed by the seedlings could be partially released from organic amendments incorporated in the substrate but a top dress application during plant growth should be also considered for a successful organic transplant production [5]. Thus, the selection of substrate and fertilization strategy is a big challenge for organic transplant production [6]. The supplementation of diluted organic liquid fertilizers directly at the root level (fertigation) can be a good system for supplying required nutrients as it increases nutrient uptake and reduces water and nutrient loss and allows growers to fulfill specific nutritional necessities [7].

Organic seedling production could also take advantage of inoculating the substrate with biofertilizer microorganisms or plant growth-promoting microorganisms also known as microbial biostimulants [7,8]. These microorganisms can directly or indirectly affect plant growth promotion either through enhanced nutrient uptake and hormonal stimulation or counteracting plant pathogens, thus allowing a more vigorous growth and healthier plants [9]. Microbial biostimulants have been applied to many vegetable crops to improve plant growth and stress tolerance [10,11] but there is limited information on their application to organic transplant production under different organic fertigation rates. Hence, the aim of this study was to test the efficacy of microbial biostimulants inoculated in the growing media to enhance the growth and quality of lettuce seedlings fertigated with increasing rates of an organic liquid fertilizer.

2. Materials and Methods

The nursery trial was carried out in a greenhouse situated at the Department of Agricultural, Food, and Forest Sciences (SAAF-University of Palermo, Italy) (38°06′28″ N 13°21′3″ E; altitude 49 m) during spring 2021. Seeds of 'Romana bionda degli ortolani' lettuce (Vilmorin, La Ménitré, France) were sown into 36 polystyrene trays with 160 cells. Twelve trays were filled with a commercial organic substrate (Compo Bio Terriccio per Orto e Semina, COMPO Italia Srl, Cesano Maderno, Italy), other twelve trays were filled with the same substrate inoculated with 0.75 g L^{-1} of Flortis Micorrize (Orvital, Settimo Milanese, Italy), and the remaining trays were filled with the commercial substrate inoculated with 1.5 g L^{-1} of TNC Bactorr^{S13} (The Nutrient Company, Rochdale, UK). Flortis Micorrize (M) and Bactorr^{S13} (B) are commercial biostimulants: the first contains 30% of *Glomus* 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.; the second contains plant growth-promoting bacteria (1.3 × 10^8 CFU g^{-1} of *Bacillus amyloliquefaciens*, *B. brevis*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. halodenitrificans*, *B. laterosporus*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B. pasteurii*, *B. subtilis*, and *Paenibacillus polymyxa*) as well as soluble humates, natural plant hormones, amino acids, vitamins, and trace elements derived from *Ascophylum nodosum*. After sowing (18 March 2021), all the trays were kept in a dark room at 20–22 °C for three days and then were moved into the greenhouse.

Two fertigation treatments were performed after 10 (plantlets with fully expanded cotyledons and the first true leaf visible) and 20 days (plantlets with three true leaves visible) from emergence by sub-fertigating the trays with four doses (0, 7, 14 and 28 mL L^{-1}) of an organic liquid fertilizer (Organic liquid vegetable plant food, Grandiol, ASB Grünland Helmut Aurenz GmbH, Stuttgart, Germany). The organic liquid fertilizer (NK 3–4) is obtained from beet marc, contains 2.7% of organic nitrogen and 0.3% of inorganic nitrogen, 18.9% of organic C, 0.3% MgO, 0.9% Na and 0.4% S, and it is suitable for organic farming according to EC regulations. Plantlets were also sub-irrigated according to their necessity until they were ready for transplant (twice a week on average). Each tray was weighed before each sub-irrigation and after drainage of the excess water to estimate the water consumed during growth. The water use efficiency (WUE) and nitrogen use efficiency (NUE) were calculated as WUE (g DW L^{-1} H_2O) = plant dry weight (g DW)/H_2O (L), and NUE (g DW g^{-1} N) = plant total dry weight (g DW)/supplied N (g) (supplied N = initial N content of the substrate + N supplied with sub-fertigation).

When lettuce seedlings had a suitable size for transplanting, four replicated samples of 25 transplants randomly selected from each treatment were destructively analyzed. Transplants were divided into roots, stems, and leaves, and weighed immediately and after drying to constant weight at 85 °C to measure the fresh and dry biomass. Soon after sampling, the leaf characteristics of each transplant were evaluated. The color components L*, a*, and b* were measured with a colorimeter (CR-400, Minolta corporation, Ltd., Osaka, Japan) on the upper part of 2 leaves, randomly selected for each seedling, and were used to calculate hue angle ($h° = 180° + \arctan(b^*/a^*)$) and chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$). Leaf area was measured with ImageJ 1.52a software (National Institutes of Health, Bethesda, MD, USA) on digital images obtained by scanning the leaves of each seedling at 300 dpi (Epson Perfection 4180 Photo, Seiko Epson Corp., Suwa, Japan). The specific leaf area was also calculated (SLA cm^2 g^{-1} DW) as leaf area/leaf dry weight.

The experimental design consisted of four replicates for each combination of microbial biostimulants and organic fertigation rates, randomly assigned in four blocks. The effect of microbial biostimulants and organic fertigation rates on lettuce seedlings (25 seedlings for each replicate) was evaluated by performing a two-way ANOVA. The least significant differences (LSD) test at $p \leq 5\%$ was applied to compare the mean values and to detect the significant differences among treatments and the significant interactions between factors.

3. Results and Discussion

The management of nutrient supply to seedlings can lead to improvements in seedling vigor and establishment in the field. The level of mineral fertilizer supplied to lettuce seedlings can influence their growth and vigor [3]. Nutrient availability, nitrogen above all, can be difficult to manage in an organic cultivation system and especially in an organic nursery where seedlings have a short cultivation period and specific nutrient needs [12].

Lettuce plantlets emerged 4, 7 and 8 days after sowing, for M, B and control, respectively. Seedlings were all ready for transplant (5–6 true leaves) after 33 d from sowing.

Plant height varied according to organic fertilization rates but with some differences due to microbial biostimulants (Table 1). Unfertigated control seedlings (10.7 cm) were slightly shorter than those treated with microbial biostimulants. The height of control seedling decreased when increasing liquid organic fertilizer concentration down to 7.6 cm, whereas B-treated seedling height was not significantly affected up to 14 mL L^{-1} and dropped down to 8.7 cm with 28 mL L^{-1} of organic liquid fertilizer (OLF). M-treated seedlings increased their height when increasing organic fertigation rate up to 14 L^{-1} and maintained a significantly higher height with 28 mL L^{-1} OLF compared to the other treatments. Stem diameter recorded small variation in control seedlings (3.4 mm on average) and was higher than 3.7 mm in the seedlings inoculated with B and fertigated with 0, 7 or 14 mL L^{-1} OLF and in those inoculated with M and fertigated with 14 and 28 mL L^{-1} OLF (Table 1).

Table 1. Effects of the microbial biostimulant treatment (C, not treated; B, Bactor[S13]; M, Micorrize) and the organic fertigation rates (ml L^{-1} of organic liquid fertilizer—OLF) on morphophysiological parameters of lettuce seedlings.

Source of Variance		Seedling Height (cm)	Stem Diameter (mm)	Seedling Fresh Weight (g FW) Total	Roots	Stem	Leaves	Seedling Dry Weight (mg DW) Total	Roots	Stem	Leaves	Dry Matter (%)	WUE (g DW L^{-1} H_2O)	NUE (g DW g^{-1} N)
Treatment														
C		[z] 9.5	3.43	1.22	0.18	0.09 b	0.95	79.6	14.6	4.7 b	60.3	6.6	3.0	15.4
B		11.7	3.63	1.67	0.23	0.13 a	1.32	96.7	16.4	5.9 a	74.5	5.9	3.0	19.0
M		12.2	3.64	1.67	0.24	0.11 ab	1.31	96.6	18.1	5.5 a	73.0	5.8	3.1	18.6
OLF (ml L^{-1})														
0		11.6	3.47	1.47	0.23	0.08 b	1.15	84.6	15.3	4.4 b	64.9	5.8	2.7	47.0
7		12.0	3.62	1.61	0.21	0.10 ab	1.29	90.8	14.2	4.8 b	71.8	5.7	2.9	11.8
14		11.8	3.70	1.70	0.23	0.13 a	1.34	100.6	18.1	6.3 a	76.2	6.0	3.4	8.1
28		9.2	3.49	1.29	0.19	0.12 a	0.99	88.1	17.9	5.8 a	64.3	6.9	3.1	3.7
Treatment × OLF														
C	0	10.7 bc	3.31 bc	1.14 d	0.15 cd	0.08	0.92 cd	67.3 d	9.2 c	3.8	54.3 c	5.9 cd	2.3 d	37.4 b
	7	10.4 c	3.60 b	1.21 d	0.14 d	0.08	0.99 cd	75.8 cd	10.0 bc	4.3	61.5 bc	6.3 bc	2.7 c	11.2 cd
	14	9.4 cd	3.52 b	1.42 c	0.25 ab	0.10	1.07 c	93.9 b	19.2 ab	5.5	69.3 b	6.6 b	3.8 a	8.9 d
	28	7.6 d	3.30 bc	1.10 d	0.19 c	0.09	0.82 d	81.5 c	20.0 ab	5.3	56.3 c	7.5 a	3.3 b	4.0 e
B	0	12.4 ab	3.70 ab	1.74 b	0.29 a	0.12	1.34 b	93.3 b	17.5 ab	4.5	71.3 b	5.4 d	2.8 c	51.8 a

	7	13.0 a	3.89 ab	1.95 a	0.25 ab	0.13	1.57 a	107.0 a	17.5 ab	6.0	83.5 a	5.5 d	3.2 b	13.5 c
	14	12.7 ab	3.76 ab	1.82 ab	0.22 bc	0.15	1.45 ab	103.0 ab	17.5 ab	6.5	79.0 ab	5.7 cd	3.2 b	7.7 d
	28	8.7 d	3.18 c	1.19 d	0.15 cd	0.11	0.93 cd	83.7 c	12.9 bc	6.5	64.3 bc	7.0 ab	2.7 c	3.1 e
M	0	11.8 b	3.41 bc	1.54 c	0.25 ab	0.06	1.18 bc	93.2 b	19.2 ab	5.0	69.0 b	6.1 c	3.1 b	51.8 a
	7	12.6 ab	3.37 bc	1.67 bc	0.24 b	0.10	1.33 b	89.5 bc	15.0 b	4.3	70.3 b	5.4 d	2.6 c	10.8 cd
	14	13.4 a	3.81 ab	1.87 ab	0.22 bc	0.14	1.51 ab	104.8 a	17.5 ab	7.0	80.3 ab	5.6 cd	3.3 b	7.8 d
	28	11.2 bc	3.98 a	1.60 bc	0.24 b	0.14	1.22 bc	99.1 ab	20.8 a	5.8	72.5 ab	6.2 bc	3.3 b	4.1 e
Significance [x]														
Treatment		***	**	***	***	**	***	***	**	**	***	***	ns	***
OLF		***	*	***	***	*	***	***	**	***	***	***	***	***
Treatment × OLF		***	***	***	***	ns	***	**	***	ns	*	***	***	***

[z] Each value is the mean of 4 replicated samples of 25 seedlings each. For each factor, values in a column followed by the same letter are not significantly different, according to the least significant differences (LSD) test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$. WUE: water use efficiency; NUE: nitrogen use efficiency.

The increase nutrient availability can influence seedling growth, but taller seedlings are often wicker and softer and more susceptible to diseases and transplant shock [13,14]. Many vegetable seedlings should be short and stocky to have a more efficient use of the greenhouse space and to ameliorate their shipment and mechanical transplanting [15].

The seedlings fresh weight (FW) was influenced by the interaction between the microbial biostimulants and the organic fertigation rates showing a significant quadratic trend in the seedling inoculated with microbial biostimulants (Table 1). The total fresh biomass of control seedlings increased significantly only supplementing 14 mL L^{-1} OLF. B-treated and M-treated seedlings had a significantly higher total fresh weight than control at every fertigation level except when B seedlings were fertigated with 28 mL L^{-1} OLF.

The total dry biomass (DW) of control seedling ranged from 67.3 to 93.9 mg DW for 0 and 14 mL L^{-1} OLF, respectively (Table 1). The inoculation with the microbial biostimulants significantly increased the dry weight of the non-fertigated seedlings (93.2 mg DW on average). B-treated seedlings had the highest dry biomass accumulation when fertigated with 7 mL L^{-1} OLF (107.0 mg DW) whereas M-treated seedlings showed the highest biomass accumulation with 14 mL L^{-1} OLF (104.8 mg DW). The dry matter percentage of the untreated seedlings increased linearly when increasing OLF concentration from 5.9% (0 mL L^{-1}) to 7.5% (28 mL L^{-1}) (Table 1). The seedlings treated with microbial biostimulants maintained lower values of dry matter percentages even when increasing OLF rate except when B seedlings were fertigated with 28 mL L^{-1} OLF.

A higher biomass content in well-developed seedlings can reduce the time from transplant to production and positively influence the yield and quality of lettuce [14]. The use of microbial biostimulant modified lettuce transplant growth and its response to organic fertigation. Root biomass was enhanced by microbial biostimulants even at low fertigation rates. Root growth is crucial to lower transplant shock and seedling loss in the field as it is linked to pulling success [14]. Moreover, the increase of seedling shoot weight may reduce the transplant shock, and increase drought tolerance, and resistance to biotic stresses [16].

The water use efficiency of the unfertigated seedlings was 2.3 g DW L^{-1} H_2O in the untreated seedlings and was significantly higher in the seedlings inoculated with M (Table1); the highest WUE was recorded in control seedlings fertigated with 14 mL L^{-1} OLF. Compared to control, B seedlings increased WUE with 7 mL L^{-1} OLF and had a lower WUE with 28 mL L^{-1} OLF. The nitrogen use efficiency was significantly increased by the microbial biostimulant only when the seedlings were not fertigated (Table 1).

The highest leaf number of the lettuce seedlings was recorded in those inoculated with B (5.5 leaves seedling^{-1} on average) (Table 2). The seedling leafiness significantly increased when increasing OLF from 0 mL L^{-1} OLF (5.2 leaves seedling^{-1}) up to 14 mL L^{-1} OLF (5.6 leaves seedling^{-1}) and then decreased with 28 mL L^{-1} OLF. The total leaf area of the seedlings was affected in different ways by the organic fertilizer levels according to the microbial biostimulant treatments (Table 2). Control seedling leaf area slightly increased by increasing the concentration of OLF up to 14 mL L^{-1} OLF but significantly reduced their total leaf area with the highest OLF concentration compared to 7 and 14 mL L^{-1} OLF. The inoculation of the substrate with microbial biostimulants was effective in improving the total leaf area of lettuce seedlings grown without fertigation or even when increasing the fertigation rate up to 14 mL L^{-1} OLF for B and up to 28 mL L^{-1} OLF for M. Leaf thickness and dry biomass distribution in the leaves can be evaluated through the specific leaf area (SLA). The effect of organic liquid fertilizer concentration on SLA followed a negative trend in control seedlings lowering from 829.9 to 626.2 cm^2 g DW^{-1} (Table 2). This negative effect was not recorded in the seedling inoculated with M and was found in those inoculated with B only when fertigating with 28 mL L^{-1} OLF.

The joint use of microbial biostimulants and organic fertigation increased the total leaf area and WUE of the seedlings. Thus, the increase in biomass accumulation could be ascribed to the wider photosynthetic area and to the higher availability of CO_2 through the open stomata. Plant growth-promoting microorganisms may improve transpiration and increase the photosynthetic rate [17–19], leading to an increase in dry matter accumulation and WUE.

Table 2. Effects of the microbial biostimulant treatment (C, not treated; B, Bactor^{S13}; M, Micorrize) and the nursery organic fertigation rates (ml L^{-1} of organic liquid fertilizer—OLF) on the leaf characteristics of lettuce seedlings.

Source of Variance		Number of Leaves		Leaf Area (cm^2 Seedling^{-1})		SLA (cm^2 g DW^{-1})		L*		Chroma		Hue°	
Treatment													
C		[z] 5.2	c	42.6		708.1		55.2		41.1		120.7	
B		5.5	a	59.2		790.6		55.9		42.0		120.8	
M		5.4	b	59.5		815.1		56.5		42.6		120.8	
OLF (ml L^{-1})													
0		5.2	c	53.4		823.1		56.3		42.2		120.4	
7		5.3	b	57.7		800.1		56.5		43.1		120.5	
14		5.6	a	59.8		780.0		56.1		41.9		120.7	
28		5.4	b	44.1		682.0		54.7		40.4		121.4	
Treatment × OLF													
C	0	5.0		43.6	cd	829.9	ab	55.2	bc	41.4	bc	120.8	b
	7	5.1		44.9	c	729.9	b	55.3	bc	41.7	bc	120.9	b
	14	5.5		46.7	c	673.4	bc	56.0	bc	40.6	c	120.3	b
	28	5.3		35.3	d	626.2	c	54.4	c	40.6	c	121.0	ab
B	0	5.4		60.2	ab	845.9	a	56.2	b	42.9	ab	120.4	b
	7	5.6		69.6	a	834.6	a	56.3	b	43.2	ab	120.7	b
	14	5.7		65.5	ab	829.0	a	56.0	bc	42.2	bc	121.0	b
	28	5.4		41.5	cd	653.1	bc	55.0	bc	39.9	c	121.0	ab
M	0	5.1		56.5	b	820.4	a	57.3	ab	42.4	b	120.1	b
	7	5.3		58.7	b	835.7	a	57.8	a	44.3	a	119.9	b
	14	5.6		67.1	ab	837.5	a	56.4	ab	42.9	ab	120.8	b
	28	5.4		55.5	b	766.7	ab	54.6	c	40.6	c	122.2	a
Significance [x]													
Treatment		**		***		***		***		***		ns	
OLF		***		***		***		***		***		**	
Treatment × OLF		ns		***		**		*		*		**	

[z] Each value is the mean of 4 replicated samples of 25 seedlings each. For each factor, values in a column followed by the same letter are not significantly different, according to the least significant differences (LSD) test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Leaf color can be and index of nutritional and stress status of the plants. It showed small variation as a function of microbial biostimulants and organic liquid fertilizer rates. Control seedling leaves did not significantly modify their color when increasing fertigation concentration, but a trend in color variation could be found with the highest OLF concentration that slightly increased the hue angle and reduced L* and chroma. A similar trend was also found for the seedlings inoculated with B, whereas M-treated seedlings showed wider and significant increases of the hue angle with 28 mL L^{-1} OLF, the highest values of L* and chroma with 7 mL L^{-1} OLF that dropped down to the level recorded in the control with 28 mL L^{-1} OLF (Table 2).

The results showed that the organic liquid fertilizer levels supplied to lettuce seedlings can influence their growth and vigor. Organic fertigation, especially with 14 mL L^{-1} of OFL, improved WUE, dry matter percentage and biomass accumulation of the control seedlings, but negatively affected their height with the highest fertigation rate. The use of the microbial biostimulants modified seedling growth and its response to organic liquid fertilizer levels. They had a growth-promoting effect on the unfertilized seedlings and modified the response of lettuce seedlings to organic fertigation but to different extents for TNC Bactorr[S13] and Flortis Micorizze.

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