EFFECT OF MICROBIAL BIOSTIMULANTS AND ORGANIC FERTIGATION ON NURSERY PRODUCTION OF LETTUCE TRANSPLANTS

Alessandro Miceli *, Alessandra Moncada, Alessandro Esposito and Filippo Vetrano

Dipartimento Scienze Agrarie, Alimentari e Forestali
Università di Palermo, ITALY
*alessandro.miceli@unipa.it
AIM OF THE STUDY
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.
Material 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) (C)

• Twelve trays were filled with the same substrate inoculated with 0.75 g L\(^{-1}\) of Flortis Micorrize (Orvital, Settimo Milanese, Italy) (M)

• Twelve trays were filled with the commercial substrate inoculated with 1.5 g L\(^{-1}\) of TNC Bactorr\(^{S13}\) (The Nutrient Company, Rochdale, UK) (B)
Material and Methods

Flortis Micorrize (Orvital, Settimo Milanese, Italy)

contains 30% of Glomus spp., $1.24 \times 10^8$ CFU g$^{-1}$ of Agrobacterium radiobacter, Bacillus subtilis, Streptomyces spp. and $3 \times 10^5$ CFU g$^{-1}$ of Thricoderma spp.

TNC Bactorr$^{S13}$ (The Nutrient Company, Rochdale, UK)

contains plant growth-promoting bacteria ($1.3 \times 10^8$ CFU g$^{-1}$ of Bacillus amyloliquefaciens, B. brevis, B. circulans, B. coagulans, B. firmus, B. halodenitrificans, B. laterosporus, 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.
Material and Methods

• 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 (OLF) (Organic liquid vegetable plant food, Grandiol, ASB Grünland Helmut Aurenz GmbH, Stuttgart, Germany).

• Plantlets were also sub-irrigated according to their necessity until they were ready for transplant (twice a week on average).

• 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.
Material and Methods

- The water use efficiency (WUE) and nitrogen use efficiency (NUE) were calculated as:
  
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  \begin{align*}
  \text{WUE (g DW L}^{-1} \text{H}_2\text{O)} &= \frac{\text{plant dry weight (g DW)}}{\text{H}_2\text{O (L)}} \\
  \text{NUE (g DW g}^{-1} \text{N)} &= \frac{\text{plant total dry weight (g DW)}}{\text{supplied N (g)}}
  \end{align*}
  \]

  (supplied N = initial N content of the substrate + N supplied with subfertigation).

- When lettuce seedlings had a suitable size for transplanting (33 days after sowing), 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 (leaf number, leaf color components, leaf area and specific leaf area).
Material and Methods

• 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.
RESULTS
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.

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

- 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.
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.

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.

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).

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\(^{-1}\))

• 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.
The water use efficiency of the unfertigated seedlings was 2.4 g DW L\(^{-1}\) H\(_2\)O in the untreated seedlings and was significantly higher in the seedlings inoculated with M.

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.
Nitrogen Use Efficiency

- The nitrogen use efficiency was significantly increased by the microbial biostimulant only when the seedlings were not fertigated.
The highest leaf number of the lettuce seedlings was recorded in those inoculated with B (5.5 leaves seedling$^{-1}$ on average).

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.

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.
Specific Leaf Area

- The effect of organic liquid fertilizer concentration on SLA followed a negative trend in control seedlings lowering from 829.9 to 626.2 cm² g DW⁻¹.
- 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.
Conclusions

• 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.

• Microbial biostimulants 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 Bactorri²¹³ and Flortis Micorizze.