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ORIGINAL PAPER

Organo-mineral Fertilizer Containing Struvite from Liquid Digestate for *Cucurbita pepo* L. Seedling Production

Sara Melito[1,2] · Domenico Ronga[3] · Daniele Marceddu[1] · Nomiki I. Kallikazarou[4] · Maria G. Antoniou[4] · Vittoria Giannini[2,5,6]

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Abstract

The increasing demand for sustainable fertilizers has made phosphorus recovery from waste a win–win solution. The present study shows the effects of two different types of organo-mineral fertilizers, derived from liquid digestate, on *Cucurbita pepo* L. (zucchini) seed germination and seedling growth. Organo-mineral fertilizers (OMF) were obtained from two biogas plants—one in Cyprus and one in Sardinia. In Cyprus, it was extracted from the digestate of mixed agricultural by-products. In Sardinia, it was extracted from the digestate of bovine slurry. Different treatment levels, compared with the traditional fertilizers, were applied in a nursery: (i) business-as-usual (0.04 g of N, 0.03 g of P_2O_5, 0.07 of K_2O per seedling), (ii) half business-as-usual level, (iii) double business-as-usual level. Agronomic, physiological, and quality parameters were assessed. At the germination level, comparable results were shown for traditional fertilizers and OMF treatments. A significant rate-response effect was observed for leaf number and area. Cyprus OMF and traditional fertilizer showed a comparable trend with the highest value at the double business-as-usual level or fertilizer. A similar trend was observed for other biometric parameters and for fresh and dry biomasses. The highest total polyphenol and anthocyanin values were recorded under Cyprus OMF and traditional fertilizer treatments at the business-as-usual level. Cyprus OMF at the highest level (2BAU, business-as-usual) presents a comparable effect on seedling growth and to traditional fertilizers probably due to the use of different agro-industrial by-products and suggesting its potential use as an alternative nutrient supply.

Keywords *Cucurbita pepo* L. · Morphological Response · Fertilizer · Struvite · Germination · Seedling Growth

1 Introduction

Intensive production of crops requires high inputs of fertilizers to meet food and feed needs (Vaneeckhaute et al. 2013). A recent study estimates that N, P_2O_5, and K_2O fertilizer requirements are as high as 110.00, 47.0, and 37.5 billion tons (Mt) per year worldwide, respectively (Spanoghe et al. 2020). More particularly, phosphorus (P) is one of the macro-nutrients that supports plant growth owing to its fundamental role in many physiological processes (Jouhet et al. 2007; Mission et al. 2005). Many soils, used for agricultural purposes, are naturally lacking biologically available P (Barber 1995). Such a deficiency is usually overcome by applying phosphate fertilizers (Cordell and Neset 2014). However, in some soils, the available P in the circulating soil solution may be not enough for plant growth due to the soil's chemical characteristics; in fact, P availability is affected by pH and the presence of Al and Fe oxides and hydroxides (Shen et al. 2011). The characteristics of the microbiome

✉ Domenico Ronga
dronga@unisa.it

1 Department of Agricultural Sciences, University of Sassari, Sassari, Italy

2 Desertification Research Centre (NRD), University of Sassari, Sassari, Italy

3 Pharmacy Department, University of Salerno, Fisciano, Italy

4 Department of Chemical Engineering, Cyprus University of Technology, Limassol, Cyprus

5 Department of Agronomy, Food, Natural Resources, Animals and Environment—DAFNAE, University of Padua, Agripolis Campus, Legnaro, Italy

6 Department of Land, Environment, Agriculture and Forestry-TESAF, University of Padua, Agripolis Campus, Legnaro, Italy

community can also reduce P availability for plants (Balemi and Negisho 2012; Sanyal and De Datta 1991).

At the same time, the P requirements of intensive agricultural and vegetable cropping systems are compensated by applying large amounts of fertilizers principally derived from the unsustainable exploitation of rock phosphate (Dumas et al. 2011). Nowadays, the main sources of mineral P are bedrocks (Smith et al. 2021) that represent non-renewable resources and manure and organic residues to a lesser extent. Considering that the rate of P extraction is higher than its replenishment rate (Smil 2000) and that the demand for P fertilizers has been predicted to increase by up to 9.2 billion tons by 2050 (Gerland et al. 2014), the identification of new sources of P is under special focus.

Struvite—a triple salt of unimolecular concentrations of ammonium, phosphate, and magnesium ($MgNH_4PO_4 \cdot 6H_2O$)—has the potential to become a valuable fertilizer. It results from the crystallization and precipitation of N and P with the presence of Mg, from different types of wastewaters and treatment steps (e.g., sludges, swine slurry, anaerobic digester side streams), in the form of a slow-release fertilizer (Uludag-Demirer et al. 2005). Various properties of struvite can affect its performance as a fertilizer. For instance, crystal size can affect the dissolution rate of struvite in soil (Degryse et al. 2017). Additionally, purity can have a significant impact on plant growth. Hall et al. (2020) found that the application of high-purity struvite fertilizers to acidic soils resulted in higher plant phosphorus availability compared to lower-purity struvite fertilizers and triple superphosphate. In addition, struvite presents a low solubility in a pH-neutral solution that makes it an interesting slow-release fertilizer that can be easily applied directly near the plant without the risk of root-burning in case of overdoses (Shu et al 2006). Struvite crystallization as a resource recovery process from animal manure could be a win–win solution. It contributes to the re-use of N and P and reduces the risk of nutrient surplus in the environment, while reducing the demand for additional conventional fertilizers made from non-renewable sources. In addition, struvite is also arguably easier to handle and transport than manure and other organic P fertilizers. A few studies have addressed the effectiveness of struvite fertilizer derived from different agriculture by-products. P from swine struvite and mineral P (P_2O_5) was taken up at similar rates for canola (*Brassica napus* L.) production, but the biomass yield per unit of P was lower under struvite treatment than under the mineral one, mainly in the early stages of canola development (maybe caused by the initial low struvite solubility) and in subalkaline soil (Ackerman et al. 2013). In another study, it was reported that struvite from cow urine improved *Vigna radiata* L. growth (Prabhu and Mutnuri 2014). Based on these data, we hypothesize that organo-mineral fertilizers containing struvite precipitates produced from different types of agricultural by-products and by two different pilot plants might have different agronomic performances in nursery productions. To test this hypothesis, we focused on *Cucurbita pepo* L. seed germination and seedling growth.

Cucurbita pepo L. is one of the most cultivated vegetable crops with a high economic value. It is mainly cropped in open fields (Kumar et al. 2009) in temperate and sub-tropical regions (Abd El-Mageed et al. 2016). Considering the world trends in terms of production volumes, the first country is China (7.4 M metric tons) while Italy ranks tenth (600.4 K metric tons) with an increasing volume (+ 5%) in the last year (Tridge 2022). High productivity can be achieved in greenhouses, where the controlled environmental conditions can double zucchini productivity compared to open-field conditions (Lucio et al. 2015). Nowadays, zucchini is usually cultivated in two phases: the first phase takes place in the greenhouse up to the two–three-true-leave stage, and the second phase takes place in the field (Milc et al. 2016). Moreover, vegetable production systems have been changed to increase yield and quality, leading to improved sustainability. In horticultural production, one of the most important sectors is seedling production in nurseries (Ronga et al. 2021). To the best of our knowledge, limited studies were conducted on the impact of organo-mineral fertilizers containing struvite on zucchini germination, growth and development, and pigment production.

The objective of this study was to determine the effectiveness of two kinds of organo-mineral fertilizers (OMF) containing struvite derived from two different sources of liquid digestates (mixed-waste digestate and bovine digestate, respectively) on zucchini seed germination and seedling growth under nursery conditions. Furthermore, zucchini responses to different concentrations of OMF-containing struvite were investigated and compared with traditional fertilizers.

2 Materials and Methods

2.1 Experimental Setup

The experiment was carried out in a nursery under natural light conditions at the Vivaio Peterle di Fabio Peterle & C., located in Arborea (Oristano, IT) (39° 45′ 24″ N; 8° 34′ 09″ E). During the experiment (April–May 2021), air temperature was between 17.0 and 20.5 °C, the average air humidity was 80.9%, and solar radiation was 87.63 W m^{-2}. Galatea F1 seeds (Enza Zaden seed company) selected for their medium-early summer cycle and resistance to Px/ZYMV/PRSV/WMV viruses were sown and grown in a plug tray (53 × 33 × 4 cm; 84 holes of 38 mm diameter each) filled with peat (Klasman). The peat was a mixture of 60% blonde/40% dark peat with PGmix 12–14-18 fertilization

(0.6 kg m^{-3}). The trays were placed in a growth chamber at 20 °C in the dark for 48 h immediately after sowing, and then in a greenhouse for 4 weeks.

2.2 Nutrient Supply

One type of OMF containing struvite was produced from a pilot biogas plant in Sardinia from the digestate of bovine manure (StS, treatment B), and the other type was produced on another pilot biogas plant in Cyprus from the ultra-filtered digestate of mixed livestock (pig slurry 50%, chicken manure 25%) and cheese whey wastewater (25%) (StC, treatment C). The compositions of the OMF are shown in Table 1.

The following macronutrients were supplied, per seedling, throughout the growth cycle: 0.04 g of N, 0.03 g of P_2O_5, and 0.07 g of K_2O. These amounts were the business-as-usual (BAU) scenario of the hosting nursery, corresponding to the fertigation treatment A (mineral fertilizers (ammonium nitrate, potassium nitrate, and urea phosphate) applied by fertigation).

The amount of OMF was based on the zucchini total P requirement for growth under BAU conditions. The amounts of N and K provided by each type of OMF were derived from their content, and the remaining N and K requirements of zucchini were met with traditional mineral fertilizers: One 33 for N and Phosfone 22 for P, purchased by Nutrien (Italy), and Kalisop for K, purchased by Agricola Internazionale (Italy). The nutrients of the treatment based on traditional mineral fertilizer (treatment D) were supplied with One 33, Kalisop, and Phosfone 22.

For treatments B and C, the traditional fertilizers and OMF were mixed with peat before sowing. For treatment D, only traditional fertilizers were mixed with peat before sowing.

The nutrients of the fertigation treatment (treatment A) were supplied daily by the irrigation system throughout the cultivation phase. Each seedling was irrigated with a total amount of 12.6 L of well water throughout the 4 weeks of the production period. Three different levels of nutrition were tested for each treatment: BAU, ½BAU (half the BAU nutrients), and 2BAU (twice the BAU nutrients). All the abovementioned treatments were performed in comparison with control plants, unfertilized, grown in peat, and only irrigated with well water (treatment E). Table 2 summarizes the treatments, their different levels, and the corresponding amounts of fertilizers.

Table 1 Chemical and microbiological analysis of organo-mineral fertilizers containing struvite from Sardinia (StS) and Cyprus (StC)

Parameters	StS	StC
pH	10.07	10.90
Total organic carbon (%)	24.9	14.9
Ammonium nitrogen ($N\text{-}NH_4$) (%)	1.58	4.90
Total Kjeldahl nitrogen (TKN) (%)	3.35	4.50
Total phosphorus (P) (%)	5.00	14.05
Water-soluble P (%)	0.56	0.25
Phosphorus pentoxide (P_2O_5) soluble in citric acid (%)	10.40	22.54
Total magnesium (Mg) (%)	4.24	7.88
Magnesium oxide (MgO) soluble in water (%)	0.40	0.18
Total potassium (K) (%)	0.99	0.68
Potassium oxide (K_2O) soluble in water (%)	0.93	0.58
Total calcium (Ca) (%)	1.11	0.02
Total sodium (Na) (%)	1.09	3.73
Total cadmium (Cd) (mg kg^{-1})	< 0.4	< 0.2
Total chromium (Cr) (mg kg^{-1})	5.84	< 1
Total copper (Cu) (mg kg^{-1})	24.63	7.53
Total nickel (Ni) (mg kg^{-1})	< 0.4	< 2
Total lead (Pb) (mg kg^{-1})	< 10	< 5
Total zinc (Zn) (mg kg^{-1})	165.33	21.1

2.3 Germination Parameters, Biometric Measurements, and Biomass Evaluation

To monitor the effect of the investigated treatment on germination, different parameters were recorded daily until 11 days after sowing (DAS). The following parameters were calculated according to Giannini et al. (2021): the germination percentage (*G*), the mean germination time (MGT), the synchrony of the germination process (*Z*), and the time to 90% germination (T90).

G (Eq. 1) was calculated as follows:

$$G = \frac{\sum_{i=1}^{k} n_i}{N} \times 100 \quad (1)$$

where n_i is the number of seeds germinated at interval i, and *N* is the total number of germinated seeds.

MGT (Eq. 2) was calculated as follows:

$$MGT = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \quad (2)$$

where $n_i t_i$ is the product of seeds germinated at interval i with the corresponding time interval.

Z (Eq. 3) was calculated as follows:

$$Z = \frac{\sum_{i=1}^{k} C_{ni,2}}{C_{\sum_{i=1}^{k} ni,2}} \quad (3)$$

where $C_{ni,2}$ is the combination of the seeds germinated in the ith time, two by two, and ranging between 0 (when at least

Table 2 Treatments, treatment levels, and nutrient supplies per treatment. The experiment was performed using four types of nutrient supplies: organo-mineral fertilizers containing struvite (from Sardinia and Cyprus, Table 1), fertigation solutions, mineral fertilizer, and control (no nutrient supply). N, P, and K were supplemented in different amounts according to the nutrient schedule and the nutrient level (BAU, ½BAU, 2BAU). *BAU*, business-as-usual; *OMF*, organo-mineral fertilizer

Treatment	Treatment level	Nutrient supply type	Struvite origin	Struvite amount per tray (g)	P supply per tray (g)	N supply per tray (g)	K supply per tray (g)
A	BAU	Fertigation solution	-	-	11.2	10.3	12.4
	2BAU	Fertigation solution	-	-	22.3	20.6	24.8
	½BAU	Fertigation solution	-	-	5.6	5.1	6.2
B	BAU	OMF-struvite	Sardinia	31.9	-	7.5	9.8
	2BAU	OMF-struvite	Sardinia	63.8	-	14.9	19.7
	½BAU	OMF-struvite	Sardinia	16.0	-	3.7	4.9
C	BAU	OMF-struvite	Cyprus	7.6	-	9.2	12.3
	2BAU	OMF-struvite	Cyprus	15.3	-	18.5	24.6
	½BAU	OMF-struvite	Cyprus	3.8	-	4.6	6.2
D	BAU	Mineral	-	-	11.2	10.3	12.4
	2BAU	Mineral	-	-	22.3	20.6	24.8
	½BAU	Mineral	-	-	5.6	5.1	6.2
E		Unfertilized	-	-	-	-	-

two seeds germinated, one at each time point) and 1 (when the germination of all seeds occurred at the same time).

T90 (Eq. 4) was calculated as follows:

$$T_{90} = \frac{t_i + \left\{ \left(\frac{N}{2} \right) - n_i \right\} (t_j - t_i)}{(n_j - n_i)} \quad (4)$$

where t_i is the time interval corresponding to n_i; t_j is the time interval corresponding to n_j; n_i is the cumulative number of seeds germinated < 9N/10; n_j is the cumulative number of seeds germinated > 9N/10, *N* is the total number of germinated seeds and following $n_i < 9N/10 < n_j$.

To monitor seedling growth and development, the following biometric parameters were recorded once a week after germination until each seedling was ready for transplanting, i.e., at 3/4 true leaves, and the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) stages 13–14: plant height (PH, cm), number of leaves (NL, n.), and root collar diameter (CD, mm). At the transplanting stage, the following parameters were recorded by destructive sampling: main root length (RL, cm), leaf area (LA, cm^2) including the total number of leaves per plant, and the ratio of the leaf area over the number of leaves per plant (LA/nL, cm^2). LA was estimated using a planimeter (LI-COR, model 3100 area meter). Five replicates per treatment level were used for all the abovementioned parameters. Aerial and root fresh weights (AFW, g; RFW, g) and dry weights (ADW; RDW) were recorded to assess plant biomass. ADW and RDW were obtained by drying the plant tissues at 60 °C for 72 h.

2.4 Estimated Leaf Chlorophyll Content, Total Phenols, Anthocyanin, Flavonoids

The leaf chlorophyll content was estimated indirectly using a SPAD 502 Plus, Konica Minolta instrument, by selecting the middle part of five fully expanded leaves of five plants per treatment (Uddling et al. 2007). SPAD indexes were recorded 12, 14, 17, and 25 days after sowing (DAS). Total phenols (TP) were directly determined by measuring extracts from leaf disk samples (100 mg FW) at an absorbance of 320 nm (A_{320}) (Maggini et al. 2018). The results were expressed as mg of gallic acid equivalents (GAE) g^{-1} FW. Two technical replicates were processed for each biological replicate. Anthocyanin (An) and flavonol glycosides (Fl) were determined following the protocol of Hradzina et al. (1982). The abovementioned secondary metabolites were extracted from 100-mg (FW) leaf disk samples using an 80:17.7:2.3 v/v/v mixture of MeOH:dH_2O:37% HCl (0.1 mL mg^{-1} FW), keeping the samples at – 20 °C for 2 days and renewing the solution after 1 day. Two technical replicates were assessed. The An concentration was determined at A_{530} against a calibration curve drawn with cyanidin 3-galactoside chloride (Merck KGaA, Darmstadt, Germany). The results were expressed as mg cyanidin-3-glucoside g^{-1} FW. Fl were determined at A_{360} against a calibration curve drawn with a pure sample of quercetin 3-glucoside. The results were expressed as mg quercetin-3-glucoside g^{-1} FW.

2.5 Statistics

A completely randomized design with five treatments (A, B, C, D, E) and three levels (BAU, ½BAU, 2BAU) was used with 5 replicates. Statistical analyses were performed using RStudio software (Team 2014) (packages lme4, emmeans, multicomp). Given the heteroscedasticity of *G*, MGT, T90, LA, and NL after Bartlett's test, these parameters were processed using a generalized linear model with a quasi-Poisson distribution using a logit link function. *Z* was processed using a generalized linear model with a quasi-binomial distribution using a logit link function. All other parameters (PH, CD, RL, AFW, RFW, ADW, RDW) were normally distributed and were subjected to an analysis of variance (ANOVA). The significance of the differences between the mean values of the treatments was assessed using Tukey's test at $P < 0.05$.

3 Results

3.1 Effects of the Fertilizers on Germination Parameters

The effects of different fertilizer treatments and the level of nutrition supply on germination (*G*) are shown in Fig. 1. *G* was equal to 100% under treatments E and A regardless of the level, under treatments D- and B-BAU, and under treatment C-½BAU. The lowest value was recorded under treatment D-2BAU but was still high (86.3%).

MGT ranged between 2.91 and 6.84 days (Fig. 2). The lowest value was recorded under treatment A-2BAU, and the highest under treatment D-2BAU. The highest values under treatments A and B were recorded at ½BAU, followed by BAU and 2BAU. Conversely, the highest values were recorded at 2BAU under treatments C and D, followed by BAU and ½BAU. MGT under treatment E was similar to MGT under treatments C- and D-½BAU and B-BAU and -2BAU. MGT altogether followed a similar trend to T90 (time to 90% germination, Supplementary Fig. 1).

The synchrony of the germination process (*Z*) was also evaluated (Fig. 3). Overall, a low level of *Z* was observed under all treatments. The lowest *Z* was found under treatment C-2BAU, and the highest under treatment A-2BAU. *Z* under treatments A- and B-BAU and -2BAU was higher than under treatments C- and D-BAU and -2BAU.

3.2 Effect of Fertilizers on the Number of Leaves and Leaf Area

Figure 4 depicts the trends of NL and LA per leaf (LA/nL) 25 DAS. The lowest NL was found under treatment E, and the highest under treatment C-2BAU, closely followed by D-2BAU. NL increased slightly under treatments A- and B-½BAU but decreased at BAU and 2BAU. A different trend was observed under treatments C and D: NL increased from ½BAU to 2BAU and reached the highest significant value (5.5 leaves) under treatment C-2BAU (Fig. 4 a).

LA/nL was deeply affected by the treatment and the treatment level. Overall, treatments A and B showed no statistical difference in terms of LA/nL and resulted in a lower LA/nL than treatments C and D whatever the fertilizer level. LA/nL under treatments A and B was comparable to LA/nL under treatment E, while treatments C and D resulted in a significant increase of LA/nL compared to the other treatments, with the lowest value recorded at ½BAU (Fig. 4b).

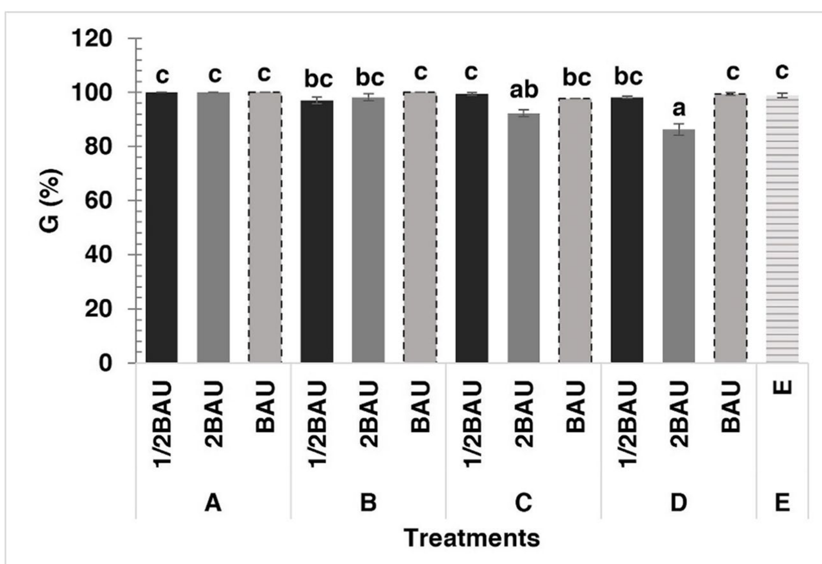

Fig. 1 Effect of different nutrient supplies on the germination (*G*; %) of *Cucurbita pepo* L. seeds. (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Vertical bars represent standard errors, and different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's test). BAU, business-as-usual

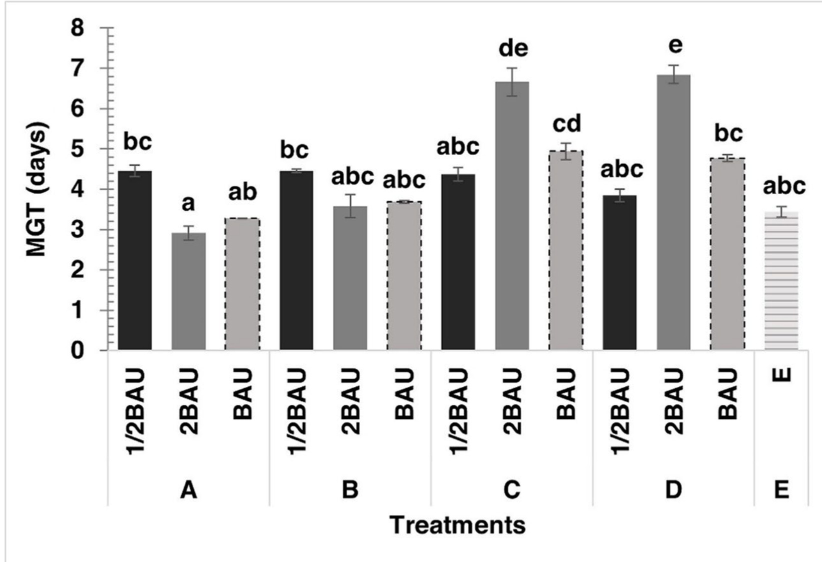

Fig. 2 Effect of different nutrient supplies on the mean germination time (MGT) of *Cucurbita pepo* seeds. (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Vertical bars represent standard errors, and different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's Test). BAU, business-as-usual

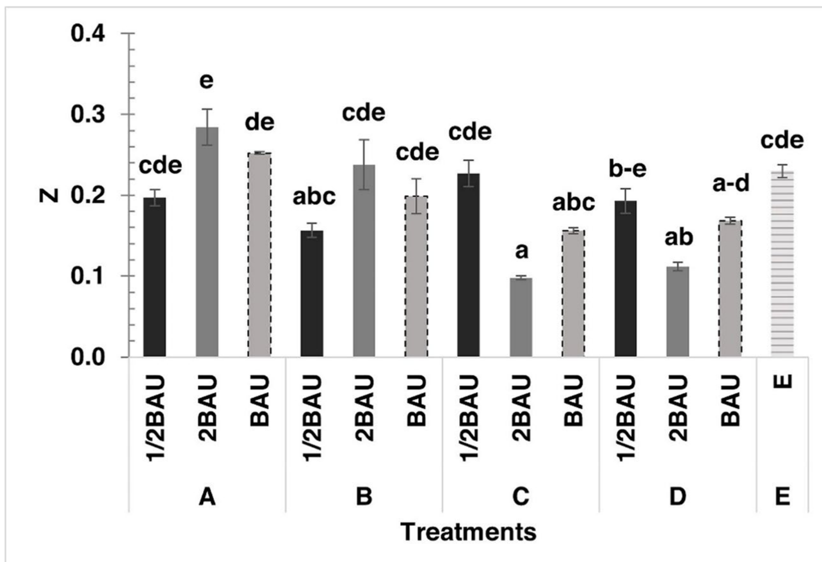

Fig. 3 Effect of different nutrient supplies on the synchrony of *Cucurbita pepo* seed germination (*Z*). (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Vertical bars represent standard errors, and different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's test). BAU, business-as-usual

3.3 Seedling Growth and Biomass

The ANOVA results of biometric parameters (RL, CD) and biomass (AFW, RFW, ADW, RDW) recorded 25 DAS are reported in Table 3.

PH, ADW, RFW, and the SPAD index were affected by the treatment × treatment level interaction. RL and CD were significantly affected by the treatments. The highest RL was found under treatment C-2BAU, and the shortest under treatment A-½BAU. Similar values were found at the other treatment levels. The lowest CD values were recorded at all levels of treatments A and E, and the highest under treatments C-BAU and -2BAU and D-2BAU. As for seedling biomass, the fresh weight of the aerial part (AFW) was significantly affected by the investigated treatments. The highest AFW was found under treatment C-2BAU and was twice as high or even higher than under treatments A, B, and E. The highest RFW was recorded under treatment C-BAU, and the lowest under treatment D-BAU, while the values at the other treatment levels were comparable among them. As for ADW, the highest values were recorded at BAU under treatments A and B, versus 2BAU under treatments D and C. Finally, the control treatment (E) showed the lowest ADW, and RDW was not affected by the treatment level (Table 4).

3.4 Biometric Growth and SPAD Index Trend Throughout the Experiment

The data of the seedling growth and development were analyzed to compare the treatment levels at given dates

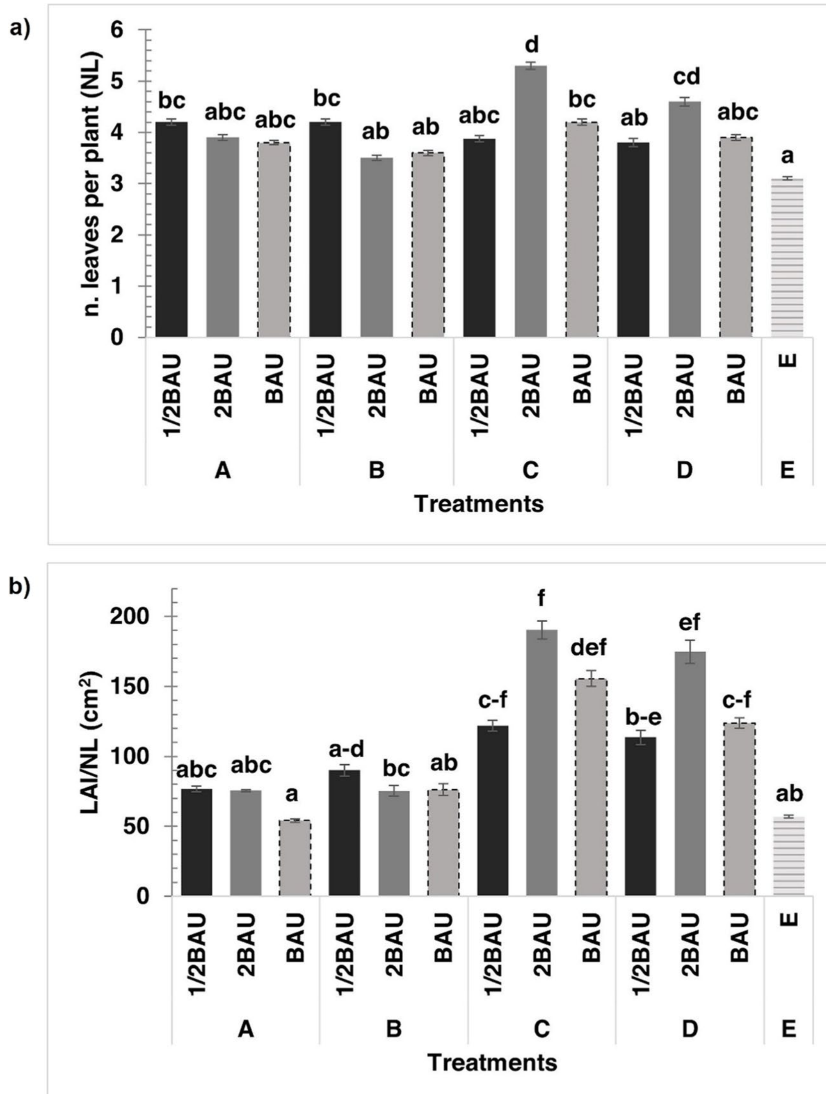

Fig. 4 Effect of different nutrient supplies on the number of leaves (NL) (**a**) and leaf area/number of leaves (LA/nL) (cm^2) (**b**) in *Cucurbita pepo* at the sampling relieve (25 DAS). (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Vertical bars represent standard errors, and different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's Test). BAU, business-as-usual

Table 3 Significance of the fertilizer treatment and its level. ANOVA was performed on biometric (*PH*, plant height; *RL*, root length; *CD*, crown diameter), biomass (*AFW*, aerial part fresh weight; *RFW*, root fresh weight; *ADW*, aerial part dry weight; *RDW*, root dry weight), and SPAD index parameters

	PH	RL	CD	AFW	RFW	ADW	RDW	SPAD
Treatment	***	**	***	***	***	***	NS	***
Level	*	NS	NS	NS	*	**	NS	**
Treat. x Level	***	NS	NS	NS	***	*	NS	***

NS, not significant; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

and different dates within the same treatment (Figs. 5 and 6). As expected, PH increased under almost all treatments and levels from 12 to 25 DAS, and over time under treatments A and B. In both cases, the highest reference point for each observation date was recorded at BAU, followed by 2BAU and then ½BAU (Fig. 5).

The only exception was under treatment B 25 DAS, when PH at BAU was similar to PH at ½BAU and significantly lower than at 2BAU. Under treatment C, the highest PH at each date was at ½BAU, followed by BAU, and then 2BAU. Under treatment D, the lowest PH at each date was recorded at 2BAU. On the other hand, the recorded PHs at BAU and

Table 4 Effect of different fertilizers on biometric and biomass parameters of *Cucurbita pepo* seedlings. Fertigation solution (A), organo-mineral fertilizer from Sardinia and Cyprus (B and C, respectively), mineral fertilizer (D), and control condition were tested at three levels (1/2BAU, BAU, 2BAU). Root length (RL) and collar diameter (CD) were used as morphological traits. Aerial fresh and dry weight (AFW and ADW, respectively) and root fresh and dry weight (RDW and RDW, respectively) were used as biomass indices. *BAU*, business-as-usual

Code	Level	RL (cm)	CD (mm)	AFW (g)	RFW (g)	ADW (g)	RDW (g)
A	½BAU	14.33 ± 3.32a	4.16 ± 0.32a	4.16 ± 1.11a	0.81 ± 0.31a	0.34 ± 0.10abc	0.06 ± 0.02
	BAU	16.94 ± 3.357ab	4.42 ± 0.36a	4.82 ± 0.78abc	0.85 ± 0.22a	0.49 ± 0.08c	0.07 ± 0.01
	2BAU	15.07 ± 3.91ab	4.32 ± 0.36a	4.46 ± 0.74a	0.90 ± 0.26ab	0.41 ± 0.05bc	0.07 ± 0.02
B	½BAU	16.13 ± 4.57ab	4.84 ± 0.49ab	4.85 ± 1.31abc	1.18 ± 0.39abcd	0.33 ± 0.10abc	0.06 ± 0.02
	BAU	16.55 ± 5.033ab	4.77 ± 0.48ab	4.14 ± 1.32a	1.31 ± 0.59abcd	0.34 ± 0.07abc	0.09 ± 0.07
	2BAU	16.62 ± 2.88ab	4.71 ± 0.66ab	4.64 ± 2.06ab	1.08 ± 0.36abc	0.29 ± 0.10ab	0.07 ± 0.03
C	½BAU	16.6 ± 2.20ab	4.75 ± 0.79ab	6.44 ± 2.56abcd	1.10 ± 0.31abcd	0.29 ± 0.11ab	0.06 ± 0.01
	BAU	19.6 ± 2.55ab	5.53 ± 0.76b	7.77 ± 2.81 cd	1.68 ± 0.64d	0.38 ± 0.15abc	0.08 ± 0.04
	2BAU	20.02 ± 2.70b	5.35 ± 0.52b	8.69 ± 2.42d	1.48 ± 0.27bcd	0.45 ± 0.14bc	0.17 ± 0.03
D	½BAU	17.74 ± 3.51ab	4.73 ± 0.59ab	5.93 ± 1.76abcd	0.98 ± 0.32abc	0.35 ± 0.10abc	0.13 ± 0.02
	BAU	15.46 ± 2.9ab	4.80 ± 0.58ab	6.41 ± 1.75abcd	0.86 ± 0.22a	0.38 ± 0.09abc	0.06 ± 0.01
	2BAU	19.42 ± 1.72ab	5.30 ± 0.52b	7.72 ± 3.40bcd	1.57 ± 0.43 cd	0.42 ± 0.11bc	0.08 ± 0.03
E	-	16.24 ± 4.03ab	4.12 ± 0.25a	3.40 ± 0.68a	0.95 ± 0.13ab	0.23 ± 0.05a	0.06 ± 0.01

½BAU did not differ statistically, except at the last observation date (25 DAS). Finally, PH increased significantly over time under treatment E.

The same data analysis approach was applied to the SPAD index. SPAD index increased under all treatments from 12 to 14 DAS (Fig. 6).

Under treatment A, the highest SPAD index was recorded at 14 DAS at all BAU conditions and then decreased to 17 DAS and 25 DAS. Under treatment B, the SPAD index increased from 14 to 17 DAS at BAU and ½BAU, while it decreased at 2BAU. The lowest SPAD indexes were observed at BAU and 2BAU 25 DAS and were the lowest recorded under treatment B at any time point. Under treatment C, the SPAD index decreased from 14 to 17 DAS. Twenty-five DAS, SPAD index was significantly higher at 2BAU than at the other levels and was the highest recorded under treatment C. Under treatment D, the SPAD index increased at all BAU levels from 12 to 14 DAS and kept increasing slightly from 14 to 17 DAS at BAU and 2BAU but decreased at ½BAU. Finally, under treatment E, the SPAD index increased from 12 to 14 DAS and then decreased. At harvest date (25 DAS), the lowest SPAD indexes were recorded under treatments B-2BAU and -BAU and A-½BAU and the highest under treatments C- and D-2BAU.

3.5 Determination of Leaf Secondary Metabolites (Total Phenols, Flavonoids, and Anthocyanins)

The leaf contents in total phenols (TP), total flavonoids (Fl), and total anthocyanins (An) were measured at 25 DAS (Fig. 7). The highest TP level was detected under treatment C-BAU, and the lowest under treatments E and D-½BAU. The lowest Fl level was found under treatment A-2BAU, and the highest under treatment E. As for An, the highest level was recorded under treatment D-BAU, and the lowest under treatment A-2 BAU. Similarly to An, the lowest Fl level was found under treatment A-2BAU, and the highest under treatment E.

4 Discussion

In seedling nurseries, one of the main parameters for measuring plant growth, especially when novel fertilizers are used, is the germination percentage (G). In the present study, all assessed treatments induced G greater than 86.3%, hence with values above that one considered threshold values for phytotoxicity ($G = 50\%$) (Zucconi et al. 1981). Considering the BAU level, all treatments performed in the same way: they indicated no effect of OMF-containing struvite on the G of zucchini seeds. These results are consistent with those on other crops such as canola (Katanda et al. 2019) and with innovative fertilizer and substrate based on the valorization of agricultural by-products (De Falco et al. 2021; Ronga et al. 2019).

Another important parameter is the mean germination time (MGT) which serves as a proxy of seed vigor. Seeds with a low MGT are more vigorous and germinate within a shorter time (Warraich et al. 2002; Braziene et al. 2021). The investigated OMF at the BAU level performed as well as the fertigation treatments used by the nursery growers suggesting the ability of the tested OMF to replace the traditional management. We also evaluated the synchrony of the germination process (Z)—an important parameter for the

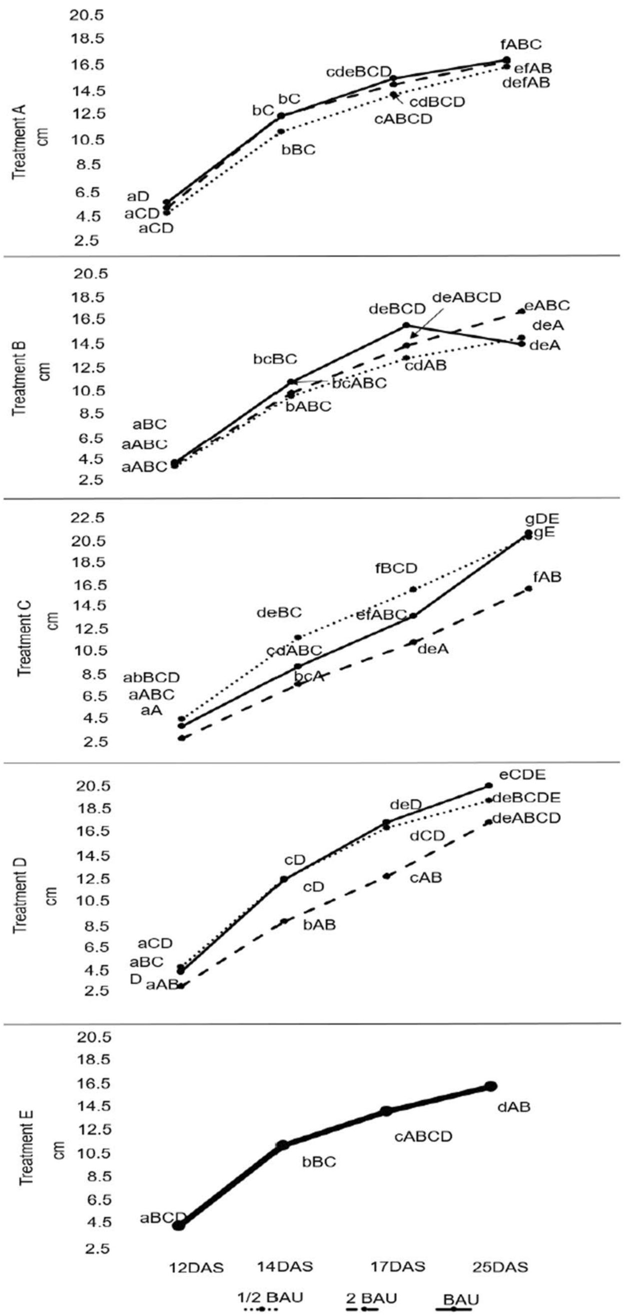

Fig. 5 Comparison of *Cucurbita pepo* seedling growth under different fertilizer and treatment levels. Seedling growth was monitored by measuring plant height (PH, cm) 12, 14, 17, and 25 DAS. (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's test) within the same treatment and level; different combinations of capital letters indicate significantly different means ($P < 0.05$, Tukey's test) among different treatment levels of the same treatment. BAU, business-as-usual

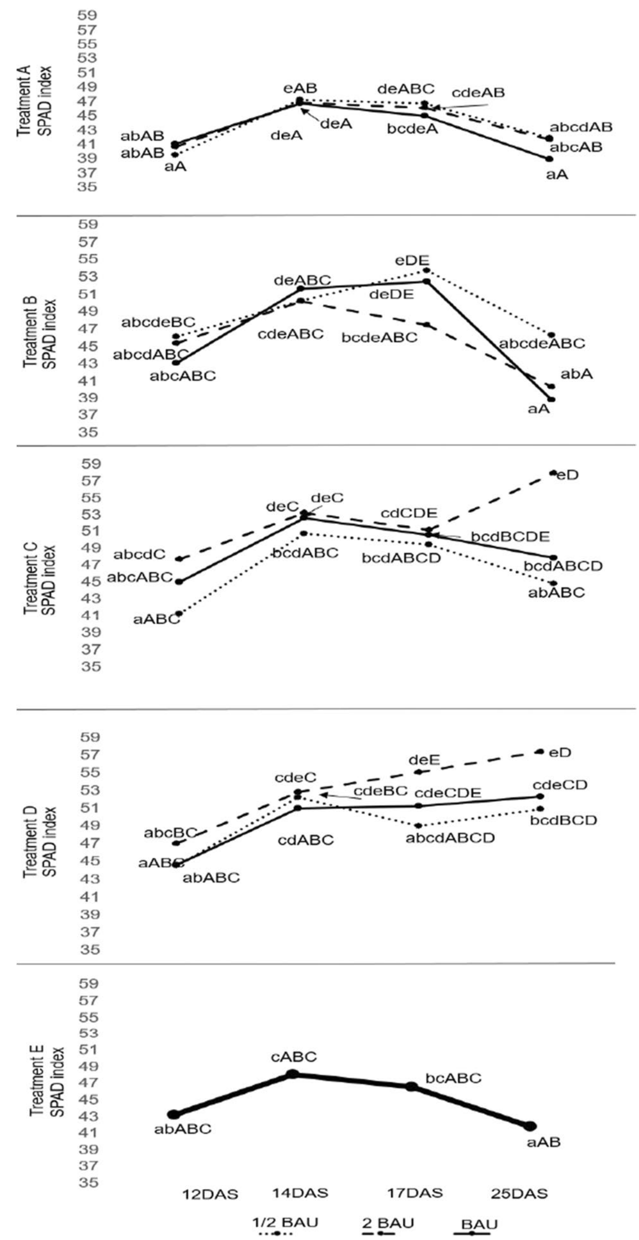

Fig. 6 Comparison of *Cucurbita pepo* seedling SPAD indexes under different fertilizer and treatment levels. SPAD indexes 12, 14, 17, and 25 DAS are shown. (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's test) within the same treatment and level; different combinations of capital letters indicate significantly different means ($P < 0.05$, Tukey's test) among different levels of a given treatment. BAU, business-as-usual

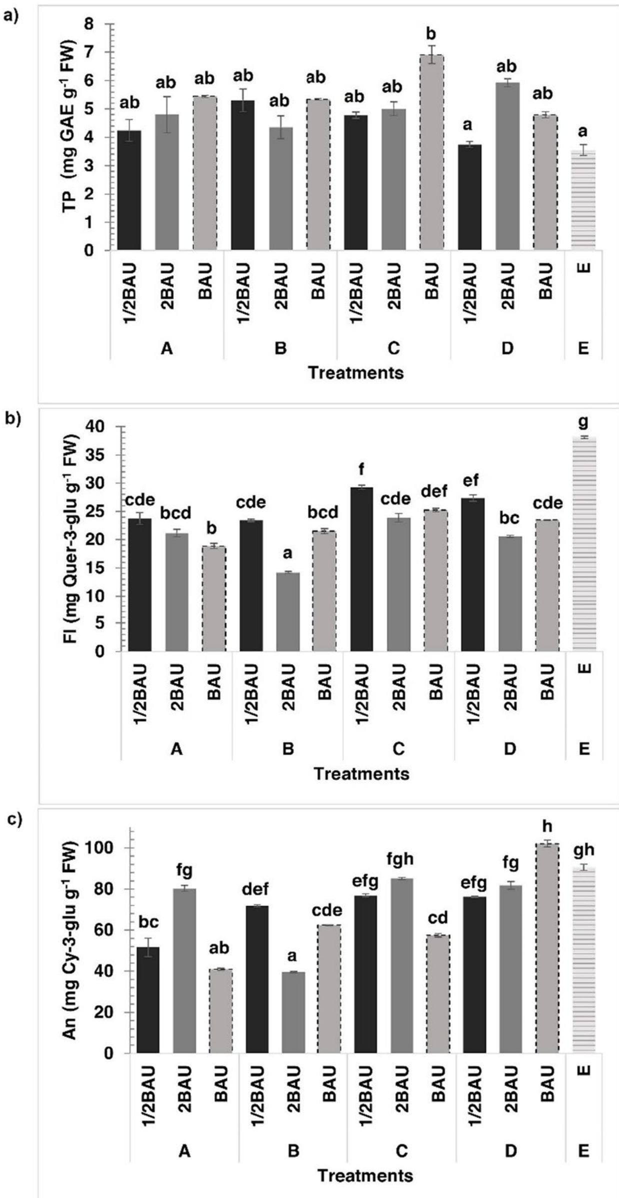

Fig. 7 Secondary metabolite contents of *Cucurbita pepo* seedling leaves. Total phenols (TP) (**a**), total flavonoids (Fl) (**b**), and total anthocyanins (An) (**c**) were measured at 25 DAS (**a**, **b**, and **c**, respectively). (A) fertigation solution; (B) organo-mineral fertilizer containing struvite from Sardinia; (C) organo-mineral fertilizer containing struvite from Cyprus; (D) mineral fertilizer; (E) water control. Different levels (½BAU, BAU, 2BAU) were tested for each treatment. Vertical bars represent standard errors, and different combinations of lower-case letters indicate significantly different means ($P < 0.05$, Tukey's test). BAU, business-as-usual

homogeneity and uniformity of seedling growth. The highest *Z* values were obtained under treatments A-BAU, B (OMF obtained in Sardina)-BAU, -2BAU, and C (OMF coming from Cyprus)-½BAU. The differences between the two types of OMF can be attributed to their different organic matter contents and water solubility of P. The low *Z* values recorded under treatment C-2BAU indicate a very low degree of synchrony of seed germination. This lack of synchrony was further confirmed by the high MGT, which indicated a low rate of germination over time. However, considering the general effects on seeds, OMF used at the BAU level and compared to treatment A performed well for all seed parameters (*G*, MGT, and *Z*) again suggesting its agronomic value, replacing the commercial traditional fertilizers and valorizing the agro-industrial by-products, for the production of *Cucurbita pepo* L. seedlings. Our results are also in accordance with those already published. In fact, as reported in the literature, organic fertilizers can increase the seed germination index, final *G*, and the seed germination rate coefficient more than synthetic fertilizers do (Aboukila et al. 2018; Deshev et al. 2020; Setti et al. 2019).

The number of leaves and leaf area are also two important parameters that reflect the stress experienced by a crop following transplanting. The number of leaves (NL) depends on several environmental factors such as the soil nutrient levels (Liu et al. 2011; Millet et al. 2019). In the present study, the highest NL was recorded under treatments C (OMF coming from Cyprus)- and D (only using traditional fertilizer)-2BAU. On the other hand, all treatments performed in the same way at the BAU level, which proves the ability of OMF-containing struvite to replace conventionally produced fertilizers also for this biometric parameter. Similar results have been recorded in maize (Liu et al. 2011) and processing tomato (Ronga et al 2019). In addition, comparable values were also obtained at ½BAU under all treatments, showing that the BAU level could be reduced without compromising plant growth. These results might be due to the dissolution of OMF-containing struvite and its ensuing availability for the crops, favored by the sub-acidic pH of the used growing media (Hertzberger et al. 2020). Seedlings were indeed grown on a dark peat substrate with a sub-acidic pH in the present study (data not shown).

LA/nL was deeply affected by the treatment and the treatment level. The highest values were recorded under treatments C (OMF coming from Cyprus)- and D (using only traditional fertilizer)-2BAU. These results might be attributed to the different feedstocks used to produce the digestates, differences in the pre-treatments of the digestates, and the design of OMF containing struvite precipitation reactor which resulted in the production of OMF of different qualities. This hypothesis is supported by the literature: struvite solubility varies depending on the struvite source (Rech et al. 2019). In addition, struvite dissolution could be driven by organic acids exuded by plant roots (Talboys et al. 2016) as well as by organic acid release by soil microorganisms (Valle et al. 2022).

The highest RL was recorded under treatment C (OMF coming from Cyprus)-2BAU, and the lowest under treatment A-½BAU. At the BAU level, all treatments performed similarly to treatment D (using only traditional fertilizer), suggesting that OMF-containing struvite could replace conventionally produced fertilizers for growing zucchini seedlings in nurseries without affecting their root growth. In addition, a treatment with a positive effect on root growth is a desirable parameter for nursery growers because seedlings with well-developed root systems better overcome transplanting stress (Eklind et al. 2001; Bignami et al. 2023).

The biomass and in particular the dry weight of seedlings is crucial for successful transplanting because it correlates with resistance to transplanting stress (Herrera et al. 2008; Ronga et al. 2021). Regarding seedling fresh biomass, the highest AFW value was recorded under treatment C (OMF coming from Cyprus)-2BAU, while the highest RFW value was recorded at the BAU level. On the other hand, a different trend was observed for ADW: the highest values were recorded under treatments A (fertirrigated seedlings) and D (only using traditional fertilizer) at whatever the level, and under treatments B (OMF produced in Sardinia)- and C (OMF coming from Cyprus)-BAU.

Our results are in agreement with those of Yetilmezsoy et al. (2009), who worked on *Lolium perenne* L. and reported that fresh and dry weight decreased when the struvite level exceeded twice the crop demand. In addition, the slow-release pattern of struvite can also ensure maximum N uptake by plants (Rahman et al. 2011; Hertzberger et al. 2020). However, variable crop responses to struvite compared to conventional P fertilizers have been reported to date. Growth of canola (*Brassica napus* L.) measured from its biomass was 28% lower under struvite fertilization than under monoammonium phosphate fertilization. However, the dry biomass of the rocket (*Eruca sativa* Mill.) increased by 488% under struvite fertilization compared with no P added (Ahmed et al. 2018).

Finally, struvite dissolution is a function not only of the pH but also of particle size (Cabeza et al. 2011; Polat and Sayan 2019), which may explain why Cyprus OMF performed better than Sardinia one in terms of AFW. In fact, Cyprus OMF contains struvite with lower particle size than Sardinia ones (data not shown). The performance of StC mimicked the behavior of a synthetic slow-release fertilizer, while the Sardinian OMF performed similarly to the conventional fertilizer treatment. However, further studies need to be conducted to confirm this hypothesis, so as to monitor nutrient release during the production of zucchini seedlings. From 12 to 25 DAS, PH increased under almost all treatments and levels. The highest values were recorded at BAU for all treatments, followed by 2BAU and ½BAU. The only exception was observed under treatment B (OMF obtained in Sardinia) 25 DAS, when the highest PH was recorded at ½BAU. These results are confirmed by the lower PH under treatment B-BAU compared to ½BAU. Our results are in agreement with those obtained from maize, in a comparison of struvite with commercial fertilizers (Rahman et al. 2013; Valle et al. 2022): the authors reported no difference between the two treatments. In addition, Valle et al. (2022) reported reduced potential P leaching losses and environmental impacts compared to the conventional fertilizers.

The SPAD index is a useful parameter to monitor the vegetative status of crops, especially in terms of nitrogen assimilation and leaf photosynthetic activity (Ronga et al. 2018a, b). The index increased under all treatments from 12 to 14 DAS. The highest value was recorded at BAU, except under treatment D (only using traditional fertilizer) (2BAU). The presence of Mg in struvite makes it an interesting alternative to traditional fertilizers for Mg-demanding crops (Gaterell et al. 2000; Arcas-Pilz et al. 2021). These results may be mainly attributed to the Mg incorporated with struvite and to a synergistic effect on P uptake (González-Ponce et al. 2009). In addition, Mg is part of the chlorophyll molecule, thereby essential for photosynthesis by enabling the activation of many plant enzymes needed for growth (Mengel and Kirkby 2004; Zepka et al. 2019). On the other hand, the behavior at 25 DAS differed, and treatments A (fertirrigated seedlings) and B (OMF obtained in Sardinia) showed the highest value at ½BAU, versus 2BAU under treatments C (OMF coming from Cyprus) and D (only using traditional fertilizer). These results might be correlated to NL, which reached the highest significant value under treatment C (OMF coming from Cyprus) -2BAU. However, more investigations are needed to clarify this aspect.

Secondary metabolites are used by plants to overcome stress (Edreva et al. 2008; Khare et al. 2020). The lowest total phenolic content was detected under treatments D (only using traditional fertilizer)-½BAU and E (unfertilized seedlings), with similar values for all the other treatment combinations except C (OMF coming from Cyprus) -BAU. This result might indicate that treatment D (only using traditional fertilizer)-½BAU was not the most appropriate strategy for producing zucchini seedlings, especially as the SPAD index was lower than at the other levels. Otherwise, the lowest An and Fl were recorded under treatment B (OMF obtained in Sardinia)-2BAU, along with the highest SPAD index, suggesting that C assimilation was mainly used to synthesize primary metabolites.

5 Conclusion

The application of two types of organo-mineral fertilizers containing struvite as potential surrogates of traditional fertilizers showed interesting effects on *Cucurbita pepo* L. seedling production, opening an interesting scenario for nursery production, and the commercialization of new organo-mineral fertilizer. The two investigated organo-mineral fertilizers, obtained with different digestates and technologies, were used at three concentrations and had significant impacts on seed germination (germination percentage, mean germination time, and synchrony) and seedling development. Application of organo-mineral fertilizers containing struvite coming from Cyprus and that one produced in Sardinia resulted in the highest average germination time at the highest organo-mineral fertilizer level (2BAU) compared to all other treatments. However as hypothesized, different digestates as well as different pilot plants can influence the agronomic performance of organo-mineral fertilizers containing struvite. In fact, the organo-mineral fertilizer coming from Cyprus at the highest level (2BAU) had a significant positive impact on almost all biometric and biomass parameters, in a similar trend to a traditional fertilizer. These results may maybe due to the lower particle size of the fertilizer coming from Cyprus and by the different organic matter contents and water solubility of P of the assessed organo-mineral fertilizers containing struvite. However, additional investigations are needed to explore the potential use of organo-mineral fertilizers containing struvite on plant productivity. In fact, might be interesting to assess the effects of organo-mineral fertilizer containing struvite not only during the nursery growth but also their agronomic performances after the seedling transplanting in the open field and the effects on fruit yield and quality, as well as the production sustainability of the organo-mineral fertilizers containing struvite from the valorization of the liquid digestate.

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Author Contribution DR contributed to the conception and design of the study. VG organized the database and performed the statistical analysis. SM and VG wrote the first draft of the manuscript. SM, VG, DM, NIK, MGA, and DR wrote sections of the manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

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Data Availability The datasets generated during and/or analyzed during the current study are not publicly available due to an agreement between partners but are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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