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Alternative Solution to Synthetic Fertilizers for the Starter Fertilization of Bread Wheat under Mediterranean Climatic Conditions

Fakir Mathlouthi [1], Roberto Ruggeri [2,*] and Francesco Rossini [2]

[1] FBSM Nanobiology, Friedrich-Ebert-Anlage 36, 60325 Frankfurt, Germany; info@fbsmnanobiology.com
[2] Department of Agriculture and Forest Sciences, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy; rossini@unitus.it
* Correspondence: r.ruggeri@unitus.it

Abstract: Under the high temporal variability of the Mediterranean climate, the prompt emergence of wheat seedlings and early vigor are pivotal attributes, positively affecting the final grain yield. Phosphorus (P) fertilization at sowing is largely used in wheat cultivation to support the early-season growth and promote a more rapid crop establishment. While conventional farming can rely on a wide variety of synthetic fertilizers, the number of options available for the starter fertilization of organic wheat is restricted and many are often unsuitable. Nanotechnology applied to fertilizers could provide a valuable means to combat this issue. This study aimed to evaluate the starter effect of a granular soil bio-enhancer (SBE) on bread wheat grown in two locations of Northern Tunisia as compared to conventional fertilization with diammonium phosphate (DAP). The SBE was obtained by physically grinding phosphate rocks at a nanoscale level and further mixing it with azotobacters. Aerial dry biomass, plant height, crop density, tiller density, leaf area index (LAI), and leaf P concentration were determined up to 70 days after emergence (DAE) to assess the early vigor of wheat seedlings. The application of SBE before sowing resulted in a greater early vigor of wheat seedlings as compared to commercial DAP over three consecutive growing seasons. Specifically, the aerial dry biomass and the plant height at 7 DAE were found to have increased by 56% and 48%, respectively. LAI at 70 DAE was enhanced by 8.5%, while moderate percentage increases were detected for both crop and tiller density. Finally, wheat plants fertilized with SBE showed a significantly higher leaf P concentration than DAP-fertilized plants in 2020 and 2021 (+21% and +32%, respectively).

Keywords: biofertilizer; early vigor; nano-fertilizer; organic farming; phosphorus; starter fertilization; wheat

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1. Introduction

Among other options, optimizing phosphorus fertilization during the crop cycle is a crucial strategy to ensure food security and quality while limiting the problem of eutrophication [1,2]. Many studies have provided evidence of the pivotal role that phosphorus (P) plays in the earliest growth stages of diverse plant species [3–5]. Distributing P fertilizers at sowing increases nutrient availability for radicles, causes positive rhizosphere chemical alterations, and reduces total nutrient losses, thus improving early growth and the use efficiency of applied nutrients [6–10]. The positive effects of starter P fertilization are even clearer in sown crops than in planted ones, because seedlings need to rapidly extend an efficient root system, especially in non-optimal environmental conditions. Synthetic fertilizers such as diammonium phosphate (DAP), monoammonium phosphate (MAP), and triple superphosphate (TSP) are the most common P sources applied in conventional farming. Conversely, soil fertility in organic farming systems relies on the use of natural sources. In recent years, organic retail sales have increased and the global organic farmland area has continuously risen, reaching 72.3 million hectares in 2019 [11]. In this context, both politicians and researchers have promoted organic farming as one of the leading tools to improve human health and environmental sustainability. While crop yield in response to the application of many organic amendments (manure, compost, crop residues, biochar, etc.) has been widely studied [12], very little research has been conducted to investigate the starter effect of fertilizers permitted in organic farming. Moreover, the scarce information that currently exists derives mainly from summer crops [13,14], because the optimal soil temperature and water content (due to the irrigation) in late spring can easily accelerate the mineralization activity of the soil's microbial community [15]. On the contrary, low temperatures and high rainfall can cause a deficiency in the availability of soil nutrients, especially during the initial growth of winter crops under organic farming conditions [16,17]. For example, the gap in grain yield between organic and conventional wheat has often been attributed to the difficulty in synchronizing the nutrient needs of crops with soil nutrient availability [18,19]. Therefore, the use of organic sources as winter crop fertilizers is a risky choice due to the complex processes that take place in the soil, such as organic matter mineralization, nitrogen immobilization, P immobilization, and turnover patterns in relation to the nutrient demands and loss pathways of crops.

In the Southern Mediterranean region, wheat-based cropping systems represent the supporting structure of farming activity. In this limiting environment, characterized by a high year-to-year climatic variability, the early growth features of wheat (germination, seedling emergence, root establishment, and early vigor) often decide the final performance of the crop [20]. Moreover, studies conducted on wheat and barley have shown that the P supply prior to the 6th week of growth affected the final grain yield much more than the P supply in later growth [9]. For all these reasons, research investigating alternative solutions to synthetic fertilizers and organic amendments for starter fertilization of wheat is not only warranted, but also highly desired.

The objective of this study was to evaluate the starter effect of a biofertilizer on bread wheat grown in Northern Tunisia, as compared to traditional fertilization with DAP.

2. Materials and Methods

2.1. Site and Experimental Design

Rain-fed field trials were carried out in Medjez el-Bab, governorate of Beja, Northern Tunisia (36°38′58″ N, 9°36′44″ E), in the 2018–2019, 2019–2020, and 2020–2021 seasons (from this point onward referred to as 2019, 2020, and 2021, respectively) and used to assess the starter effect of two fertilizers on bread wheat. Experiments were conducted simultaneously in two locations (hereinafter referred to as L1 and L2). Details of each experimental year and location are reported in Table 1. Since this study aimed to evaluate the response of bread wheat during the early growth stages, the weather data correspond to the period from sowing to the beginning of tillering (October–December).

Soil samples were collected in both locations before sowing. The samples, taken at a 0–30 cm depth, were oven dried, grounded, and then analyzed to determine their textural and chemical properties (Table 1).

At the end of summer, soil was ploughed at 30 cm depth and then harrowed to allow a proper seedbed preparation. Plots were sown with the highly productive bread wheat cultivar 'Byrsa', which is commonly grown in Northern Tunisia [21].

A randomized complete block design with three replicates was used, which resulted in 6 plots (2 fertilizers × 3 replicates) for each location and year. The experimental plots were 5 m long and 1.2 m wide (six rows spaced 0.2 m each other).

Table 1. Description of experimental seasons and locations.

	2018–2019		2019–2020		2020–2021	
Experiment Information	**L1**	**L2**	**L1**	**L2**	**L1**	**L2**
Sowing date	22 October	22 October	21 October	21 October	21 October	21 October
Preceding crop	Barley	Barley	Wheat	Wheat	Wheat	Wheat
Sowing density (seeds m^{-2})	300	300	300	300	300	300
Plot size (m^2)	6	6	6	6	6	6
Wheater data						
Total rainfall (mm)	28	31	44	49	32	36
Mean air temperature (°C)	16.3	16.0	15.7	15.2	15.0	14.7
Soil features						
Clay (Ø < 2 µm, %)	30	40				
Silt (2.0 < Ø < 20 µm, %)	64	58				
Sand (2.0 > Ø > 0.02 mm, %)	6	2				
Soil texture	Silty clay loam	Silty clay				
Available P (%)	0.94	0.91				
Total $CaCO_3$ (%)	3.73	2.91				
Organic matter (%)	1.42	2.00				
SiO_2 (%)	30.2	42.1				
Al_2O_3 (%)	18.7	23.2				
Fe_2O_3 (%)	9.19	11.4				
pH	7.45	7.32				

L1 and L2: location 1 and 2. Ø: diameter of soil particles. Weather data refer to the period October–December for each growing season.

Commercial DAP (NP 18–46) was used as a control treatment to assess the starter effect of an innovative fertilizer authorized for organic farming obtained from the treatment of a natural phosphate ore slurry. The treatment consisted of fragmenting phosphate rocks into nano particles and mixing them with azotobacters inside a colloidal reactor. This process resulted in a granular fertilizer, which was coated with two layers: a first (inner) layer, consisting of colloidal nano-calcium phosphate, and a second (outer) layer, consisting of bending azotobacters and calcium phosphate. The end result was a rounded granule that could release P, Ca, microelements, as well as N in a very controlled manner. The presence of azotobacters could classify this fertilizer as a biofertilizer/soil bio-enhancer (SBE, [22]). This product, which was formulated by the company FBSM Nanobiology and commercially known as Terios, had the following composition (according to a mass spectrometer-based analysis): total N, 5.0%; total P (P_2O_5), 15.0%; K_2O, 5.0%; CaO, 25.0%; Fe_2O_3, 0.5%; MgO, 4.0%; Mo, 10 ppm; Zn, 100 ppm; Mn, 8 ppm.

Both fertilizers were applied at sowing, with a rate of 150 kg ha^{-1} and 250 kg ha^{-1} for DAP and SBE, respectively. The resulting total P amount applied for each season was 69 kg ha^{-1} and 37.5 kg ha^{-1} for DAP and SBE, respectively.

2.2. Sampling and Measurements

In each location and experimental year, the following traits were recorded: crop density (plants m^{-2}), aerial biomass (g DM m^{-2}), plant height (mm), tiller density (n m^{-2}), leaf area index (LAI, m^2 m^{-2}), and leaf P concentration (mg g^{-1}).

Crop density was measured by counting wheat seedlings from two internal rows, one meter-long each, 14 days after emergence (14 DAE). The final stand was calculated with respect to the row spacing and the result was reported as plants per square meter.

Aerial biomass and plant height were recorded weekly, starting from the growth stage corresponding to the emergence of the first leaf (7 DAE) to the beginning of tillering (70 DAE). Fifty plants were collected for each sampling date, plot, and location. Plants were randomly chosen within plots, excluding the border rows. The aerial biomass was separated from the roots, oven-dried at 70 °C until it was a constant weight and then weighed.

The height was measured by a ruler from the ground level to the top of the seedlings prior to collection for biomass determination.

Tiller density and LAI were determined at 70 DAE, using the same plants previously collected for crop density measurement. Tillers were manually counted from each plant and the total tiller density was reported as tillers per m^{-2}. A leaf area meter (Model AM350, ADC BioScientific Ltd., Hoddesdon, UK) was used to quantify the total LAI from the collected plant material.

Leaf P concentration was analyzed at the end of the study (70 DAE) from a sample obtained by randomly collecting 50 plants from each plot. The phosphorus concentration in leaves was measured colorimetrically using a Technicon Auto Analyzer [23].

2.3. Statistical Analysis

A three-way analysis of variance (ANOVA) was performed using R (version 3.5.2) to test the main effects of the year, location, fertilizer, and their interactions [24]. Treatment means were separated using Fisher's protected least significant differences test at a probability level of 0.05.

3. Results

3.1. Crop Density, Aerial Dry Biomass Accumulation, and Plant Height

The analysis of variance results for the recorded traits are shown in Table 2. Third-order interactions were never significant, while second-order interactions were significant on a few occasions for aerial biomass and plant height.

The growing season had a significant effect on crop density (Figure 1), with 2021 showing a higher number of emerged seedlings, especially as compared to 2019 (+12.5%). As for aerial biomass and plant height (Tables 3 and 4, respectively), the best results were again recorded in 2021, with the highest increases recorded at 7 DAE (+20–30%). Although still significant, the advantage of SBE over DAP was markedly lowered at 70 DAE (+2% for aerial biomass and +6% for plant height, 2021 vs. 2019). Overall, the 2018–2019 season was the worst-performing season for all of the recorded variables.

Location influenced the accumulation of dry matter and plant height depending on the growth stage. Up to 35 DAE, the effect of the cultivation area was constantly significant only for plant height, while from 42 to 70 DAE it was constantly significant for aerial biomass and never significant for plant height (Tables 3 and 4). Overall, the best results were obtained under the L2 conditions, with the highest increase recorded at 7 DAE for both aerial biomass and plant height (+20% and +25%, respectively) (Table 3). Conversely, cultivation area did not influence the crop density (Figure 1).

The type of fertilizer used significantly affected the crop density; the aerial dry matter production; and, initially, plant height (Table 2). The results indicated that, as compared to DAP, SBE promoted crop density (Figure 1), even though the increase in the number of seedlings was relatively low (+3%). Aerial biomass production was found to be higher in the SBE-fertilized plots than in the DAP ones across each sampling date (Table 3). However, as already observed for the other factors, the highest benefit of SBE in comparison to DAP was recorded at the earliest growth stage for both aerial biomass and plant height (+56% and +48%, respectively). The difference decreased rapidly at 14 DAE (5% for aerial biomass and 11% for plant height) and then decreased slowly until 70 DAE, at which point there was almost no difference between DAP and SBE.

Table 2. Results from the analysis of variance for the recorded traits.

	Sampling Day	Crop Density	Aerial Biomass	Plant Height	Number of Tillers	LAI	Leaf P Concentr.
Year (Y)	7		ns	*			
	14	***	ns	***			
	21		*	ns			
	28		ns	***			
	35		*	***			
	42		***	***			
	49		***	***			
	56		***	***			
	63		***	***			
	70		***	***	***	***	***
Location (L)	7		ns	*			
	14	ns	***	*			
	21		*	**			
	28		ns	**			
	35		ns	***			
	42		*	ns			
	49		*	ns			
	56		***	ns			
	63		***	ns			
	70		*	ns	ns	*	**
Fertilizer (F)	7		***	***			
	14	***	***	**			
	21		***	***			
	28		**	**			
	35		***	***			
	42		**	ns			
	49		**	ns			
	56		***	ns			
	63		***	*			
	70		***	ns	**	***	***
Two-way interactions	7		ns	ns			
	14	ns	ns	ns			
	21		F × L **	ns			
	28		ns	F × L **			
	35		L × Y *	ns			
	42		ns	ns			
	49		ns	ns			
	56		F × Y *	ns			
	63		ns	F × L **			
	70		ns	ns	ns	ns	F × Y *

Levels of significance: ***—<0.001; **—<0.01; *—<0.05; ns—not significant. Other interactions are not reported, since they were not significantly different at the 0.05 probability level.

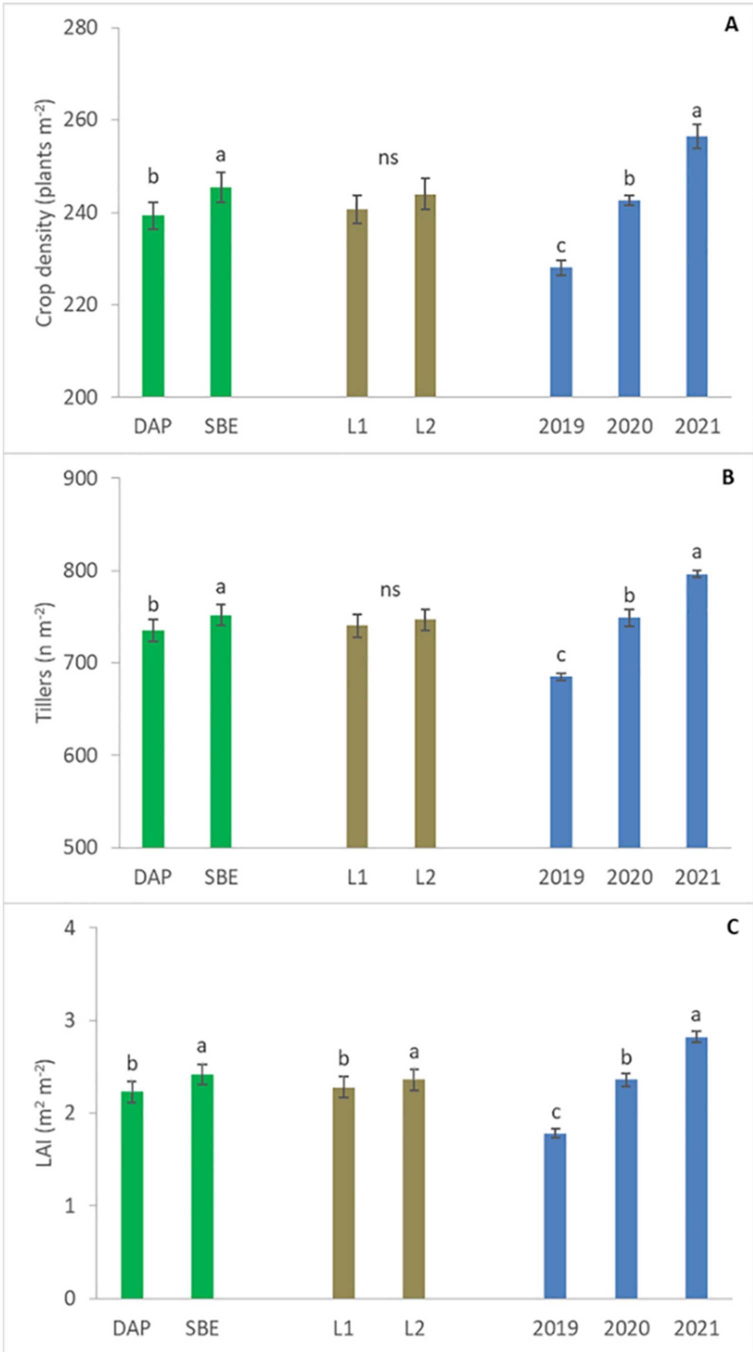

Figure 1. Crop density (**A**), tiller density (**B**), and LAI (**C**) for each applied fertilizer, location, and growing season. Error bars represent the standard error of the means; letters above the histograms correspond to the ranking of the Fisher's protected test at $p < 0.05$.

Table 3. Dynamics (from 7 to 70 DAE) of the aerial biomass production (g DM m^{-2}) for each growing season, location, and applied fertilizer.

Sampling Day (DAE)	2019	2020	2021	L1	L2	DAP	SBE
7	2.17 ns	1.92 ns	2.33 ns	1.94 ns	2.33 ns	1.67 b	2.61 a
14	14.92 ns	14.67 ns	14.42 ns	14.28 b	15.06 a	14.28 b	15.06 a
21	40.92 b	41.75 a	41.17 ab	41.00 b	41.56 a	40.39 b	42.17 a
28	92.33 ns	92.08 ns	92.75 ns	92.00 ns	92.78 ns	91.78 b	93.00 a
35	155.5 b	156.3 ab	157.5 a	156.1 ns	156.8 ns	154.8 b	158.0 a
42	239.6 c	252.5 a	245.1 b	243.2 b	248.2 a	242.4 b	249.1 a
49	321.8 c	336.0 b	348.1 a	332.4 b	338.2 a	332.2 b	338.4 a
56	512.60 c	537.9 b	555.9 a	531.1 b	539.8 a	529.2 b	541.7 a
63	708.2 c	730.6 b	746.0 a	722.3 b	734.2 a	720.1 b	736.4 a
70	844.3 c	853.3 b	861.4 a	851.0 b	855.1 a	849.1 b	856.9 a

For each factor and sampling day, means sharing the same letter were not significantly different at the 0.05 probability level. ns—not significant; DAE—days after emergence; L1 and L2—location 1 and 2; DAP—diammonium phosphate; SBE—soil bio-enhancer.

Table 4. Dynamics (from 7 to 70 DAE) of plant height (mm) for each growing season, location, and applied fertilizer.

Sampling Day (DAE)	2019	2020	2021	L1	L2	DAP	SBE
7	1.75 b	1.92 ab	2.33 a	1.78 b	2.22 a	1.61 b	2.39 a
14	9.75 b	11.17 a	11.75 a	10.44 b	11.33 a	10.33 b	11.44 a
21	23.08 ns	22.75 ns	22.58 ns	22.00 b	23.61 a	21.94 b	23.67 a
28	46.17 b	46.75 b	52.33 a	47.78 b	49.06 a	47.83 b	49.00 a
35	74.33 c	77.17 b	81.67 a	76.56 b	78.89 a	76.28 b	79.17 a
42	119.9 c	121.8 b	125.1 a	121.8 ns	122.7 ns	121.7 ns	122.8 ns
49	149.4 c	159.5 b	165.9 a	158.6 ns	157.9 ns	158.4 ns	158.1 ns
56	165.1 b	168.3 b	176.6 a	170.2 ns	169.8 ns	170.4 ns	169.6 ns
63	190.2 b	194.0 a	194.8 a	193.6 ns	192.4 ns	194.0 a	192.0 b
70	217.0 c	226.5 b	230.8 a	225.1 ns	224.5 ns	225.1 ns	224.4 ns

For each factor and sampling day, means labelled with the same letter (a, b, c) were not significantly different from each other at the 0.05 probability level. ns— not significant; DAE—days after emergence; L1 and L2—location 1 and 2; DAP—diammonium phosphate; SBE—soil bio-enhancer.

3.2. Tiller Density and Leaf Area Index (LAI)

The analysis of variance results for these traits are shown in Table 2. Interactions between factors were never significant; therefore, only the results of the main factor effects are shown (Figure 1).

The cultivation year significantly affected both tiller density and LAI at 70 DAE (Table 2). Consistent with the results of the aerial biomass production, 2021 was the most favorable year for the early growth of wheat. The highest percentage increase between years was recorded for LAI (+19% for 2021 vs. 2020 and +58% for 2021 vs. 2019). The year 2021 presented a moderate advantage for tiller density (+6% for 2021 vs. 2020 and +16% for 2021 vs. 2019).

Although significant (p = 0.037), the effect of the location on LAI was just a 3.5% increase in L2 as compared to L1. Conversely, the cultivation area did not influence the tiller density (Figure 1).

Both plant traits were significantly affected by the type of fertilizer, with SBE producing a larger effect on the LAI (+8.5% as compared to DAP) than the tiller density (+2% as compared to DAP).

3.3. Leaf P Concentration

The analysis of variance result for the leaf P concentration is shown in Table 2. The second-order interaction, fertilizer × year, was significant (p = 0.037).

Foliar P concentration increased significantly and was nearly constantly moving from 2019 to 2021 for both fertilizers (Figure 2), but with a higher yearly rate for SBE (+30%) as compared to DAP (+20%). Moreover, while wheat plants fertilized with SBE showed a significantly higher P concentration than the DAP-fertilized plants in 2020 and 2021 (+21% and +32%, respectively), the percentage increase recorded in 2019 (+14% for SBE vs. DAP) was not statistically significant (Figure 2). Consistent with the results on aerial biomass, leaf P concentration was also significantly higher under the L2 conditions as compared to the L1 conditions (data not shown).

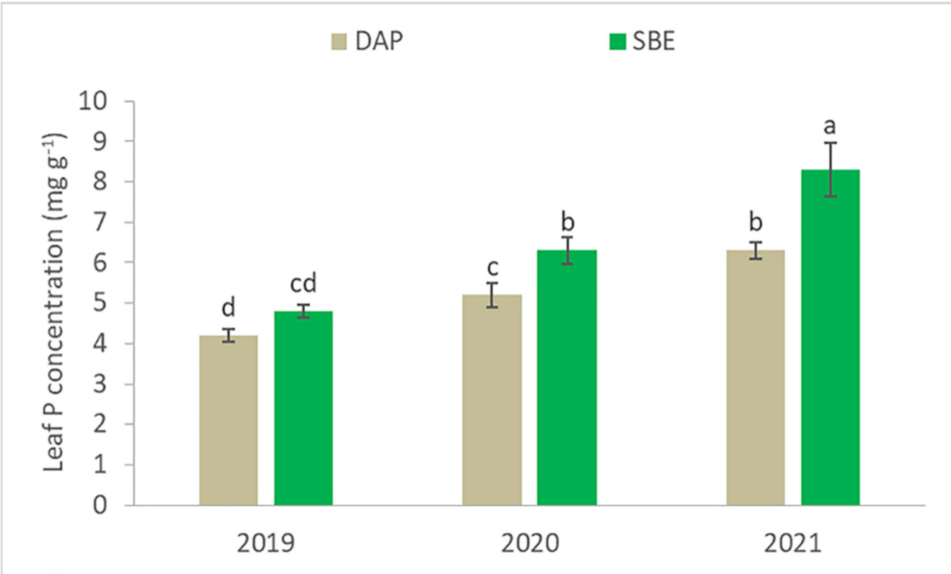

Figure 2. Leaf P concentration: fertilizer × year interaction. The error bars represent the standard error of the means and the letters above the histograms correspond to the ranking of the Fisher's protected test at $p < 0.05$.

4. Discussion

Research aiming to overcome the agronomic issues of organic farming is crucial for increasing environmental sustainability in future global agriculture. Besides the issues of pest and weed control, for which valuable responses are already provided using plant breeding in combination with agronomic tools [25–31], the availability of nutrients is the key factor in determining the yield and quality gap between conventional and organic wheat [32,33].

The results from this study suggest that a valid option, comparable to the use of DAP in conventional farming, can be applied for the starter fertilization of organic wheat under Mediterranean climatic conditions. Indeed, the distribution of SBE prior to sowing showed significantly better results than those of DAP for all recorded plant traits, especially from 7 to 14 days after emergence. At the time of writing, other authors have found that P fertilization increased P concentration and P uptake in wheat plants by 50% and 75%, respectively, as compared to an unfertilized control treatment [34]. Despite the fact that we used two P fertilizers, meaning that it was reasonable to expect little difference in the response of wheat seedlings, the fertilization with SBE resulted in a 58% and a 46% increase for aerial dry biomass production and plant height at 7 DAE, respectively, as well as having a higher crop density. This suggests that there is more rapid P availability for young seedlings when using SBE in comparison to DAP. This can be primarily attributed to the particle size in SBE granules. Even though the mechanisms are still not completely understood, the positive effect of nanoparticles (diameter < 100 nm) on germination and seedling growth seems to be due to an easier penetration in seeds and radicles, thus improving the uptake of water and nutrients [1,35–37]. Moreover, hydroxyapatite nanoparticles were recently found to strongly stimulate root elongation in tomato seedlings [38].

Crop density, fast early growth, and LAI are all plant traits related to wheat early vigor [25,39,40]. Production of a greater aerial biomass early in the growing cycle is considered of utmost importance for semi-arid Mediterranean cropping systems because it enhances uniform plant stand, accelerates establishment and canopy closure, promotes root growth, increases water- and radiation-use efficiency (RUE), and decreases soil water evaporation [39,41,42]. All these aspects contribute to the promotion of more sustainable farming systems by limiting the leakage of water and nutrients and improving weed suppression ability.

Furthermore, the water-absorbing capacity of coated fertilizer can facilitate the concentration of soil moisture on the micro-region of fertilizer dissolution, thus enhancing P diffusion and seedling uptake [43]. Recently, Santos et al. [44] evaluated the water absorption rate of a coated granule and found it to be 160% of its weight. However, other studies found evidence of a higher P release of traditional DAP as compared to coated DAP in the early stages of crop growth (up to 30 days after seeding) and an opposite behavior in the latest stages [45,46]. Consequently, it was concluded that P release from coated fertilizers is greatly dependent on coating material, thickness (number of layers), environmental conditions, and plant species.

Besides the positive effects of P nanoparticles and coating, SBE fertilization also added other macro- and micronutrients close to the seeds (not present in conventional DAP), that possibly acted as promoters of germination and initial growth. Specifically, the role of starter nutrients was recognized for Mg and Zn [47–49].

Analyzing the results at 70 DAE, SBE markedly lowered plant height and aerial dry biomass production as compared to DAP, likely as a consequence of the effect of nutrients derived from DAP fertilization. Future studies should verify if the specific characteristics of SBE (nanoparticles and coating) may have indirect positive effects beyond the early tillering stage (e.g., on grain yield, protein content, P use efficiency). Indeed, the significantly higher P concentration and LAI we found at 70 DAE for SBE (+25% and +8.5% on average, respectively), could be the trigger for positive traits affecting the final grain yield, such as new tiller emergence, increased photosynthetic area of the canopy, and higher weed competitiveness [25,50–52]. Moreover, the controlled nutrient release of coated SBE, as compared to commercial DAP, could directly influence wheat production by enhancing the plant P-use efficiency [46]. Additionally, the putative P solubilizing activity of azotobacters [22] could play an important role in maintaining high P availability in the later wheat stages (i.e., booting and heading).

In our study, we found a strong effect of the cultivation year on the measured variables. Since the air temperature during the early growth of the wheat was quite similar across the three seasons, the year-to-year variability can mainly be attributed to the difference in the rainfall amount between the sowing and early tillering stages. Adequate soil moisture at seeding, and immediately after, is essential to rapidly complete the phases of germination and seedling emergence. Considering the sowing date of the experimental plots, the precipitations that affected the earliest growth stages were those in October and November. In 2019 (the driest season), total rainfall in these two months was 10 mm for both locations, while it was three and two times more in 2020 and 2021, respectively. However, excessive soil moisture can be as detrimental as drought for seed germination and seedling emergence by causing waterlogging, seed diseases, and soil crusting. Moreover, these events are very likely to happen in soils with silt as the main fraction and a low organic matter content [53], such as those found in L1. All of this contributed to the significant differences we found in the results from 2020 and L1 with respect to 2021 and L2, as well as the significant effect of fertilizer × year interaction on leaf P concentration at tillering (70 DAE). In this regard, soil drought during the early growth stages of wheat in 2019 has clearly hindered the nutrient uptake of wheat seedlings [54], thus weakening the advantage of SBE over DAP. Finally, the constant increase in leaf P concentration we found for both fertilizers moving from 2019 to 2021 was likely due to the growing soil P availability resulting from the three years of P fertilization [55,56].

5. Conclusions

The application of the tested biofertilizer before sowing clearly promoted the early vigor of wheat seedlings when compared with commercial diammonium phosphate.

The practical consequences for Mediterranean crop management can be enhanced water- and nutrient-use efficiency, as well as higher competitiveness against weeds. These aspects take on even greater importance when artificial fertilizers and herbicides cannot be used (e.g., under organic farming conditions).

Moreover, the results from our study showed that a better performance can be achieved using less nutrients, thus improving the environmental sustainability of winter cereal production. Indeed, taking into account the grade of tested fertilizers, the application of SBE at 250 kg ha^{-1} as compared to DAP at 150 kg ha^{-1} can save about 32 kg P ha^{-1} (almost 50%) and 15 kg N ha^{-1} (54%).

Additional data are required to understand if the enhanced early vigor of wheat plants will have also positive effects on grain yield and quality at harvest.

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