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Effects of foliar fertilization of biostimulants obtained from sewage sludge on olive yield

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Abstract: The objective of this study was to investigate the effect of foliar fertilization of two biostimulants (BS1 and BS2) obtained from sewage sludge by fermentative processes using *Bacillus licheniformis* during four consecutive seasons in an olive tree crop applied at a dose of 6 L ha-1 divided into three applications (before flowering, beginning of flowering and fruit set). BS2 had a higher number of low molecular weight peptides the BS1. The contents of macro and micronutrients in leaves, photosynthetic pigments and olive yield were higher in plants fertilized with BS2 than BS1. With respect to the control treatment and for the 2017 and 2019 seasons, the olive yield increased by 20–22% in plants fertilized with BS2 and by 28–29% for the plants foliar fertilized with the BS1. For the 2018 and 2020 seasons, the olive yield increased by 35–36% in the plants fertilized with the BS1 and by 42–43% for the plants fertilized with the BS2. These results suggest that the foliar use of these biostimulants, that contain a higher number of low molecular weight peptides, could be of great interest to farmers as regards improving olive crop yield.

Subjects: Agriculture & Environmental Sciences; Agronomy; Agriculture

Keywords: Sewage sludge biostimulant; fermentation process; olive nutrition

1. Introduction

One of the most important current challenges in agriculture is to produce sufficient food to meet the growing demands of the world population while also introducing agricultural practices that are environmentally sustainable in terms of eliminating or reducing the generation of greenhouse gases, soil salinization, water eutrophication and food security problems (Duhamel & Vandenkoornhuyse, 2013; Colla & Rouphael, 2015; Ye et al. 2020).

Many scientists consider that the use of organic biostimulants could be very useful to meet these challenges (Kapoore et al., 2021; Searchinger, 2013). In addition, biostimulants obtained from agro-industrial by-products would represent a sustainable solution to the problem of waste disposal, thereby addressing the circular economy concept (or challenge) proposed by the EU to convert these wastes into new energy sources (Ávila-Pozo et al., 2021; Baglieri et al., 2014; Comission, 2016).

Obtaining biostimulants from agro-industrial and livestock wastes is a very interesting strategy because the recovery of these residues implies their elimination, and therefore a reduction in their environmental impact and also increases their value, thus coinciding with the concept of economy circular proposed by the EU to convert this waste into new sources of energy (Comission, 2016).

These biostimulants, which generally comprise peptides, amino acids, polysaccharides, humic acids, etc., are directly absorbed by the plants, thus improving the mineral nutrition of the plant and increasing the quality and productivity of the grain or fruit harvested (Colla et al., 2015; Tejada et al., 2018, 2018).

Foliar fertilization is currently considered to be a technique that contributes to sustainable and environmentally friendly agriculture since it significantly reduces the groundwater pollution caused by the application of chemical fertilizers to the soil (Tejada et al., 2018). In addition, such fertilization improves the absorption of nutrients by the crops, and consequently results in a faster response of the plant, thus meaning that lower amounts of fertilizer can be applied (Onofrei et al., 2017). Consequently, the application of biostimulants to crops by foliar application appears to be the ideal agricultural technique to ensure optimal use of the chemical constituents of these biostimulants while minimizing (and even eliminating) some environmental aspects such as the emission of greenhouse gases, eutrophication of groundwater or soil salinization.

Sewage sludge, which is characterized by having a high content of organic matter, macronutrients and micronutrients vital for plant growth, is a well-known organic residue derived from urban wastewater treatment. Consequently, sewage sludge is an important resource that has been used in agriculture (Angin et al., 2017; Eid et al., 2021). For example, Tejada et al. (2016) developed and applied by foliar application a biostimulant obtained from sewage sludge using enzymatic hydrolysis processes to a corn crop, subsequently observing that this product significantly improved the mineral nutrition of the crop as well as the yield quality of the grains obtained.

Rodríguez-Morgado et al. (2019) obtained different biostimulants from sewage sludge by fermentative processes using the bacterium *Bacillus licheniformis*. These new biostimulants had a high content of macro- and micronutrients, humic substances and low molecular weight amino acids. As far as we are aware, there are no references regarding the use of this type of biostimulant in yield crops, therefore the application thereof could be a good alternative for the use of these novel compounds.

The olive tree (*Olea europaea* L.) is a widely cultivated woody plant in the Mediterranean region that is of great economic importance since its fruits are consumed as both olive oil and as table olives (Besnard et al., 2018; Chatzistathis et al., 2020). For this reason, an understanding of the response of the crop to biostimulants obtained by fermentation processes could be of interest to farmers. As such, the objective of this study was to evaluate the effect of two biostimulants obtained from sewage sludge by fermentation processes on the olive yield of an olive grove located in a semiarid Mediterranean agro-ecosystem.

2. Material and methods

2.1. Site and biostimulants

The study was carried out in an olive crop in Espejo (Córdoba, Andalusia, Spain) (37° 40’ 51” N, 4° 33’ 13” W) during four experimental seasons. The characteristics of the olive trees found in the study area, as well as the planting density and crop management system, have been reported previously (Tejada & Benítez, 2020).

The climate in the study area is semi-arid. According to the Spanish National Weather Service (AEMET, 2021), the total annual precipitation was 341.9, 649.6, 327.2 and 447.9 mm for 2017, 2018, 2019 and 2020, respectively. The mean air temperature was 19.5, 17.3, 18.8, and 19.1 °C for 2017, 2018, 2019, and 2020, respectively.

The soil used in this study was a Calcaric Cambisol (WRB, 2014). This soil contains 331 ± 25 g kg^{-1} sand, 368 ± 17 g kg^{-1} silt and 301 ± 21 g kg^{-1} clay. The soil pH was 7.4 ± 0.2, the organic matter content was 16.2 ± 1.3 g kg^{-1} and the Kjeldahl-N content

0.71 ± 0.10 g kg^{-1}. The methodology used to determine these parameters is described elsewhere (Tejada & Benítez, 2020).

Two biostimulants obtained by fermentation processes using the bacterium *Bacillus licheniformis* ATCC 21415 and sewage sludge provided by CENTA (Seville, Spain) were used. The fermentation processes used are detailed elsewhere (Rodríguez-Morgado et al., 2019). However, in this experiment and unlike Rodríguez-Morgado et al. (2019) to obtain both experimental biostimulants, a temperature of 55 °C was used in the fermentation process with *Bacillus licheniformis* to avoid problems of contamination by microorganisms.

These biostimulants were:

1. BS1: biostimulant basically comprising bacteria + enzymes + hydrolyzed organic matter

2. BS2: biostimulant basically comprising hydrolyzed organic matter

The general properties of both experimental biostimulants are shown in Table 1. The methodology used to determine the chemical parameters that characterize these biostimulants can be found elsewhere (Rodríguez-Morgado et al., 2019).

According to Rodríguez-Morgado et al. (2015), sewage sludge were autoclaved in order to eliminate pathogens, particularly *Escherichia coli*, by thermal decay. Also, Rodríguez-Morgado et al. (2019), indicated that this thermal process improves the ability of *Bacillus licheniformis* to degrade higher molecular weight proteins.

Table 1. Chemical characteristics and protein molecular weight distribution (mean ± standard error, n = 3) of sewage sludge and the two experimental biostimulants

	SS	BS1	BS2
Organic matter (g kg^{-1})	475a ± 19	479a ± 17	473a ± 12
N (g kg^{-1})	30.7a ± 3.3	29.4a ± 2.1	29.0a ± 4.2
P (g kg^{-1})	14.1a ± 1.9	15.1a ± 2.2	16.3a ± 1.7
K (g kg^{-1})	6.4a ± 1.4	6.8a ± 1.0	6.3a ± 1.1
Ca (g kg^{-1})	44.3a ± 2.7	44.9a ± 3.9	43.7a ± 2.9
Mg (g kg^{-1})	7.5a ± 1.7	7.9a ± 1.4	7.7a ± 1.1
Fe (g kg^{-1})	17.5a ± 1.5	16.8a ± 1.9	18.1a ± 1.6
Mn (mg kg^{-1})	164a ± 16	162a ± 21	169a ± 18
Cu (mg kg^{-1})	298a ± 22	301a ± 19	311a ± 16
Zn (mg kg^{-1})	131a ± 17	127a ± 11	124a ± 20
Cd (mg kg^{-1})	3.5a ± 1.4	3.1a ± 0.9	3.3a ± 1.9
Ni (mg kg^{-1})	12.9a ± 2.7	13.8a ± 1.3	13.4a ± 1.1
Pb (mg kg^{-1})	6.3a ± 2.0	6.5a ± 1.7	6.2a ± 2.6
Protein molecular weight distribution (Da)			
> 10,000	98.2a ± 1.4	39.9b ± 2.9	34.8c ± 3.6
10,000–5000	0.70a ± 0.12	17.0b ± 3.2	16.9b ± 1.7
5000–1000	1.1a ± 0.2	11.7b ± 2.1	11.6b ± 1.7
1000–300	0.0a ± 0.0	2.7b ± 0.9	3.3b ± 1.1
< 300	0.0a ± 0.0	28.7b ± 2.9	33.4c ± 3.3

Files followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$).
SS: sewage sludge; BS1: biostimulant 1; BS2: biostimulant 2

2.2. Experimental layout and plant analysis

The experimental layout was a randomized complete block design with three treatments and three replicates per treatment. Each experimental plot consisted of 24 trees in a 6 × 4 orientation, with only the central trees being used for sampling (8 trees). The experimental treatments were:

(1) A0 treatment, plots without foliar fertilizer, control plot

(2) A1 treatment, plots foliar fertilized with BS1 at a dose of 6 L ha^{-1}

(3) A2 treatment, plots foliar fertilized with BS2 at a dose of 6 L ha^{-1}

The doses used were selected at random but were sufficient to ensure that the olive plant did not experience nutritional deficiencies during vegetative growth.

For each experimental season, these doses were divided into three applications (2 L ha^{-1} for each application), which were applied before flowering (March), at the onset of flowering (May) and at fruit set (June).

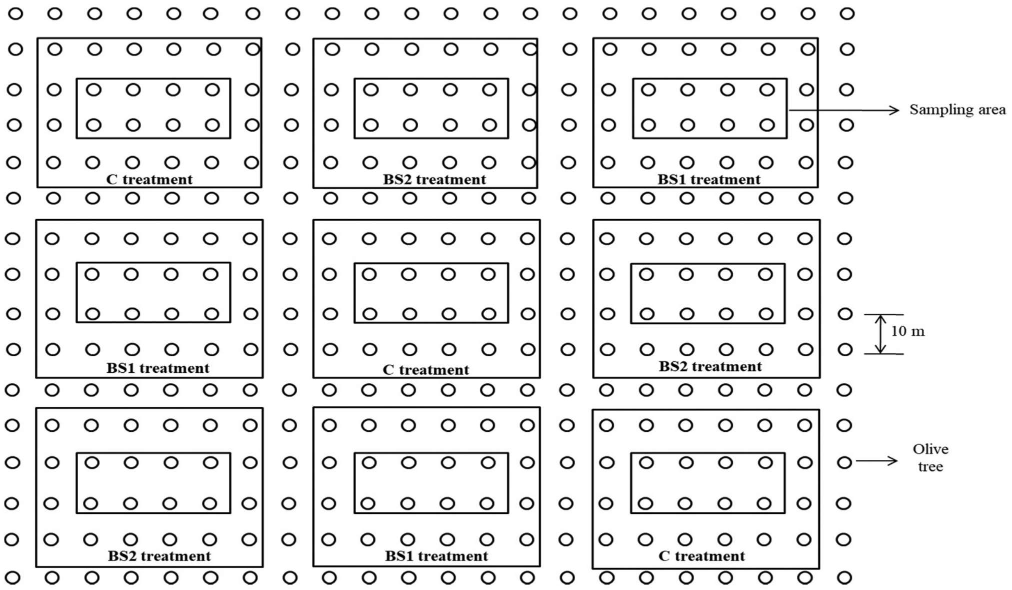
According to Tejada et al. (2016), the continuous application of experimental biostimulants is a good agricultural practice since this allows the crop to take better advantage of the nutrients over a longer period of time.

The biostimulants were applied using a manual CO_2 sprayer at a constant pressure of 0.017 MPa. They were applied early in the morning, as recommended by Tejada and Gonzalez (2003), when the stomata of the leaves began to open.

Figure 1 shows a scheme of this experimental design.

As reported by Tejada and Benítez (2020), to observe the mineral nutrition of olive trees, leaf samples (100 per season and fertilizer treatment) were collected in mid-July 2017, 2018, 2019 and 2020.

Figure 1. Design of the experimental layout.



The olive yield (kg ha^{-1}) was determined for trees harvested from each plot at the end of December (2017, 2018, 2019 and 2020). The macro- and micronutrient contents were determined for the leaves collected for each fertilizer treatment and experimental season using the methodology described in Tejada et al. (2016). For this, the leaves were washed, dried, ground and digested by wet oxidation with concentrated HNO3 under pressure in a microwave oven. The determination of macro and micronutrients (P, K, Ca, Mg, Fe, Cu, Mn and Zn) in the extracts was carried out by ICP-OES. Kjeldahl-N was determined by the MAPA method (1986) for fresh matter.

The chlorophyll A and B and carotenoid contents were also determined. These pigments were extracted with 100% acetone, as reported by Sarijeva et al. (2007), and the concentrations of the extracted pigments were calculates from the absorbance values at 664 nm. 648 nm, and 470 nm using the equations described by Lichtenthaler (1987), where:

chlorophyll a_c = 12.25 A664nm—2.79 A648nm,

chlorophyll b_c = 21.50 A648n m—- 5.10 A664nm,

carotenoids$_c$ = (1000 A470 n m—1.82 chl a_c—85.02 chl b_c)/198

A = absorvance

c = pigment concentration (µg ml^{-1} of extract)

2.3. Statistical analyses

The data obtained were subjected to a two-way analysis of variance (ANOVA) (treatment x year) using Tukey's post-hoc tests in order to detect significant differences ($p < 0.05$) between the mineral nutrition parameters of the olive tree, photosynthetic pigments and olive yield for each experimental season. The Statgraphics Plus 2.1 software package was used to carry out these statistical treatments.

3. Results

3.1. Production of biostimulants

Table 1 shows the chemical characteristics and molecular weight distribution of the proteins for both the sewage sludge and the two experimental biostimulants obtained by fermentation using the bacterium *Bacillus licheniformis*.

The statistical analysis indicates that the organic matter and macro- and micronutrient contents for the sewage sludge and the two biostimulants obtained did not differ significantly.

In contrast, the statistical analysis performed on the protein molecular mass distribution showed significant differences ($p < 0.05$). Thus, the biochemical fermentation process resulted in a decrease in the quantity of high molecular weight proteins (>10,000 Da) and a significant increase in the others protein sizes analyzed (1000–5000, 300–1000 and <300 Da, respectively) in both biostimulants. Similarly, a significant difference ($p < 0.05$) was found between the two biostimulants obtained in terms of the distribution of the molecular mass of the analyzed proteins, with a lower content of high molecular weight proteins (>10,000 Da; 12.8%) and a higher content of low molecular weight proteins (<300 Da; 14.1%) in BS2 than in BS1.

3.2. Vegetal material

Table 2 shows the foliar macro- and micronutrient contents for the four experimental seasons studied, expressed on a dry matter basis.

Table 2. Chemical composition (mean ± standard error, $n = 3$) of olive tree leaves (on a dry matter basis) for all treatments during the experiment. Columns followed by the different letter(s) are significantly different according to the Tukey test ($p < 0.05$)

	N† (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
					2017 year				
A0 treatment	9.3a ± 1.1	0.77a ± 0.05	7.1a ± 1.9	4.2a ± 1.2	15.4a± 2.1	308a± 25	4.2a ± 1.2	18.7a ± 2.1	13.5a ± 1.8
A1 treatment	11.2b ± 1.8	0.90b ± 0.09	8.9b ± 1.5	4.8a ± 1.4	18.9b ± 2.4	334b ± 21	4.9a ± 1.2	25.0b ± 2.5	15.3ab ± 1.5
A2 treatment	13.7c ± 1.5	1.1b ± 0.08	9.4b ± 1.2	5.0a ± 1.2	20.6b ± 1.9	345b ± 18	4.9a ± 1.4	29.2b ± 2.2	16.4b ± 1.1
	2018 year								
A0 treatment	11.1a ± 1.9	1.1a ± 0.2	8.9a ± 1.2	6.0a± 1.8	21.3a± 2.2	353a± 32	5.8a ± 1.1	26.9b ± 2.7	19.8a ± 1.6
A1 treatment	15.5b ± 2.1	1.7b ± 0.2	11.1b ± 2.0	6.9a ± 1.5	23.9b ± 2.1	386b ± 25	6.5a ± 1.3	31.4c ± 2.4	24.3a ± 1.9
A2 treatment	18.7c ± 2.5	1.9b ± 0.3	12.8b ± 1.9	7.1a ± 1.6	25.4b ± 2.3	441c ± 33	6.7a ± 1.2	33.8c ± 2.9	27.5b ± 1.7
	2019 year								
A0 treatment	9.0a ± 0.8	0.80a ± 0.07	7.5a ± 1.4	4.2a ± 1.0	14.9a± 2.4	312a ± 22	4.3a ± 1.0	17.7a ± 1.9	14.8a ± 1.5
A1 treatment	11.4b ± 1.9	0.92ab ± 0.07	9.1b ± 1.3	4.7a ± 1.1	18.7b± 1.9	347b ± 24	5.0a ± 1.3	25.4b ± 2.4	15.7ab ± 1.2
A2 treatment	13.5c ± 1.6	0.99b ± 0.08	9.4b ± 1.1	5.1a ± 1.3	20.0b± 2.0	359b ± 19	5.2a ± 1.3	29.7b ± 2.0	16.7b ± 1.4
	2020 year								
A0 treatment	10.8a ± 1.5	1.2a ± 0.2	9.3a ± 1.5	6.2a ± 1.4	22.4a ± 2.5	349a ± 37	5.5a± 1.4	28.3b ± 2.1	20.1a± 1.7
A1 treatment	15.1b ± 1.3	1.9b ± 0.3	11.3b ± 1.7	6.9a ± 1.3	24.1b ± 1.9	382b ± 18	6.4a ± 1.2	32.1c ± 2.7	24.1a ± 1.5
A2 treatment	18.3c ± 1.2	1.9b ± 0.2	12.5b ± 1.4	7.1a ± 1.2	25.3b ± 1.7	445c ± 24	6.6a ± 1.3	34.7c ± 2.2	27.7b ± 1.8

†Fresh matter

BS1: biostimulant 1; BS2: biostimulant 2

For the four experimental seasons, and in comparison with the control treatment, the macro- and micronutrient contents increased significantly ($p < 0.05$) in the leaves fertilized with the biostimulants by foliar application. On the other hand, differences were observed, although not significant, in terms of the macro and micronutrient values obtained for the 2017 and 2019 seasons compared to the 2018 and 2020 seasons. Thus, for the former the macro- and micronutrient contents in leaves were lower than those obtained during the 2018 and 2020 seasons, probably due to the effect of alternate bearing in the olive trees. For these 2017 and 2019 seasons, the macro- and micronutrient contents obtained were higher for plants fertilized by foliar application with BS2 than with BS1. However, significant differences ($p < 0.05$) were only observed between BS1 and BS2 treatments for N and Mn contents. Thus, N was 18.2% higher in the 2017 season and 15.6% higher in the 2019 season in the leaves of plants fertilized with BS2 rather than with BS1. The Mn content was 14.4% higher in the 2017 season and 14.5% higher in the 2019 season in the leaves of plants fertilized with BS2 rather than with BS1.

In the 2018 and 2020 seasons, the macro- and micronutrient contents of leaves were also higher in plants fertilized by foliar application with BS2 rather than with BS1. However, the statistical analysis indicated that these differences were only statistically significant ($p < 0.05$) for the N, Fe and Zn contents. Thus, N was 17.2% higher in the 2018 season and 17.5% higher in the 2020 season in the leaves of plants fertilized with BS2 rather than with BS1. Similarly, Fe was 12.5% higher in the 2018 season and 14.2% higher in the 2020 season, and Zn was 11.6% higher in the 2018 season and 13% higher in the 2020 season, in the leaves of plants fertilized with BS2 rather than with BS1.

Table 3 shows the pigments chlorophyll A, B and carotenoid contents in olive leaves for each fertilizer treatment and experimental season.

Similar to the leaf macro- and micronutrient contents, the pigment contents obtained were also lower in the 2017 and 2019 seasons than in the 2018 and 2020 seasons, probably due to the alternate bearing in the olive trees. Moreover, for all experimental seasons studied and fertilizer treatments used, the values for these pigments were higher in plants fertilized by foliar application with biostimulants than in unfertilized plants. However, these differences were only significant ($p < 0.05$) in the 2019 and 2020 seasons between chlorophyll A with treatments A1 and A2, with a higher content of this pigment being observed (16% for the 2019 season and 19.5% for the 2020 season) for A2 than for A1.

3.3. Olive yield

The type of biostimulant applied to the plants also produced significant differences in olive yield between the different fertilizer treatments and experimental seasons (Figure 2). Thus for the 2018 and 2020 seasons, the olive yield was significantly ($p < 0.05$) higher in the BS1 and BS2 amended plots than in control plots. For the 2017 and 2019 seasons, no significant differences between A2 and A3 treatments were found.

As for the macro- and micronutrient and pigment contents analyzed, the olive yield was higher in the 2018 and 2020 seasons than for the 2017 and 2019 seasons, also due to the alternate bearing in olive trees. Similarly, while for the 2017 and 2019 seasons the olive yield increased significantly ($p < 0.01$) by 22.2% and 20.3% for the A1 treatment and 28.4% and 29.3% for the A2 treatment, respectively, with respect to the control treatment, for the 2018 and 2020 seasons the olive yield increased significantly ($p < 0.01$) by 36.5% and 35.6% for the A1 treatment and 42% and 43.1% for the A2 treatment, respectively.

4. Discussion

First of all, it is necessary to highlight that the authors think that these new biostimulants obtained from sewage sludge can be used in agriculture.

In order to obtain these biostimulants, the sewage sludge has been autoclaved and then subjected to a fermentation process with *Bacillus licheniformis* at 55 °C (thermophilic anaerobic digestion), which will eliminate a large part of the possible pathogens that the untreated sludge may present., especially *Escherichia coli* (Rodríguez-Morgado et al., 2015).

On the other hand, another of the great drawbacks in the use of sewage sludge in agriculture is its heavy metal content. In this sense, and in accordance with the European Council Directive 86/278/EEC, the heavy metal content of both the untreated sludge and the biostimulants obtained from said sludge show values lower than the limiting values given by said European legislation. .

Our findings suggest that foliar application of the new biostimulants obtained from sewage sludge by fermentation processes using the bacteria *Bacillus licheniformis* has a positive effect on the mineral nutrition of the olive tree as well as on its photosynthetic pigments content and olive yield.

These results are in agreement with those obtained by other authors, who obtained an improvement in both yield and crop quality after foliar application of various biostimulants comprising a mixture of amino acids and humic substances. Thus, Tejada et al. (2016) observed an improvement in the mineral nutrition of a corn crop and the yield and quality of the corn cobs obtained after the application of various biostimulants obtained from chicken feathers and sewage sludge with a high content of humic substances and peptides. Similarly, Kandil et al. (2016) observed an increase in the yield and quality of a wheat crop when applying mixed humic acids and amino acids by foliar application.

This stimulating effect on the olive plant exerted by our experimental biostimulants is a consequence of their chemical composition, especially their content of organic matter and peptides. In this sense, there is a large amount of information about the biostimulant properties presented by both humic substances and peptides (Onofrei et al., 2017; Radkowski & Radkowska, 2018; Tejada et al., 2016, 2018).

Çelik et al. (2010), Tejada et al. (2016), and (2018)) have suggested that the foliar application of humic substances improves the permeability of the cuticle, thus favoring penetration of the different chemical compounds found in the biostimulants into plant cells. In our experiment, the two biostimulants used do not show significant differences in terms of the concentration of organic matter. Consequently, this chemical parameter cannot be responsible for the significant differences found in terms of leaf content, pigments and olive yield.

The application of protein hydrolysates improves the absorption of water and nutrients as well as N metabolism by activating various enzymes that intervene in this process, thus improving the efficiency of both macro- and micronutrients (Colla et al., 2015; El-Sanatawy et al., 2021). This enhancement of plant metabolism promotes the processes of plant respiration, photosynthesis and protein synthesis, thereby improving crop yield and quality (Kocira et al., 2020; Radkowski & Radkowska, 2018).

In our experiment, the content of low molecular weight peptides was higher in BS2 tan BS1. We think that higher content of low molecular weight peptides in BS2 is responsible for the improvement in crop mineral nutrition, photosynthetic pigment content and olive production when BS2 is applied by foliar application. This may allow the plant to more easily absorb peptides of lower molecular weight than those of higher molecular weight, thus facilitating the translocation of said peptides by the plant. This will have a more positive impact on the mineral nutrition and, therefore, crop yield.

In support of this hypothesis, it should be noted that Quartieri et al. (2022) found differences in shoot and root growth in a potted kiwi crop when biostimulants were applied at different doses

Table 3. Evolution of chlorophylls A and B and carotenoids (mean ± standard error, $n = 3$) of olive tree leaves (on a dry matter basis) for all treatments during the experiment. Columns followed by the different letter(s) are significantly different according to the Tukey test ($p < 0.05$)

	Chlorophyll A (mg g^{-1})	Chlorophyll B (mg g^{-1})	Carotenoids (mg g^{-1})
	2017 year		
A0 treatment	0.48a ± 0.08	0.19a ± 0.07	8.2a ± 0.5
A1 treatment	0.52ab ± 0.1	0.26ab ± 0.1	9.3b ± 0.8
A2 treatment	0.57b ± 0.2	0.29b ± 0.06	9.6b ± 0.7
	2018 year		
A0 treatment	0.51a ± 0.1	0.22a ± 0.07	8.8a ± 0.9
A1 treatment	0.63b ± 0.2	0.30b ± 0.06	10.1b ± 0.4
A2 treatment	0.75c ± 0.2	0.34b ± 0.1	10.9b ± 0.6
	2019 year		
A0 treatment	0.46a ± 0.1	0.21a ± 0.09	8.0a ± 0.5
A1 treatment	0.50ab ± 0.3	0.28ab ± 0.07	9.5b ± 0.8
A2 treatment	0.58b ± 0.2	0.30b ± 0.1	9.9b ± 1.0
	2020 year		
A0 treatment	0.50a ± 0.2	0.24a ± 0.05	8.6a ± 0.8
A1 treatment	0.62b ± 0.2	0.31ab ± 0.08	10.2b ± 1.0
A2 treatment	0.77c ± 0.3	0.36b ± 0.07a	11.1b ± 1.1

BS1: biostimulant 1; BS2: biostimulant 2

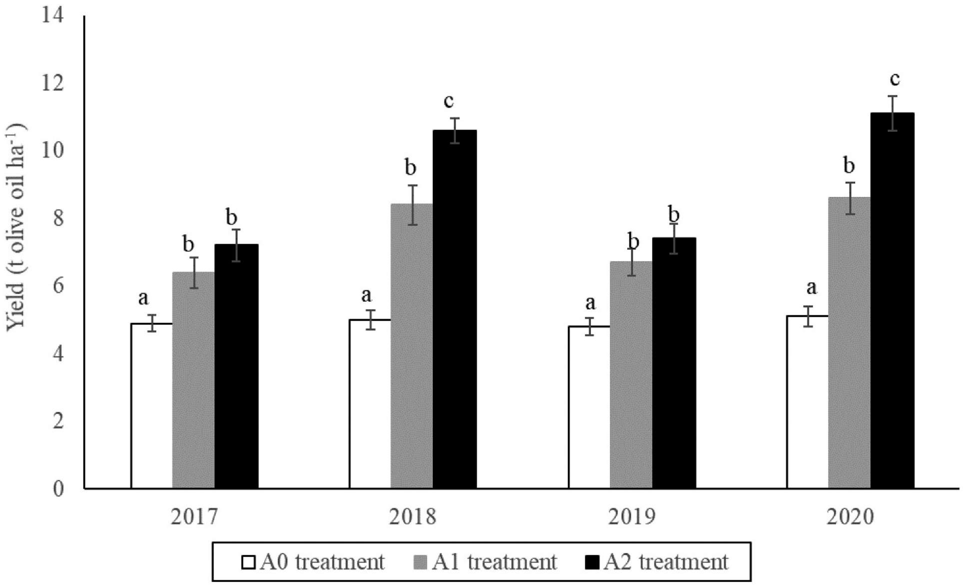


Figure 2. Olive yield during the four experimental seasons (mean ± standard error, n = 3). Columns followed by the different letter(s) are significantly different according to the Tukey test (p < 0.05).

and different molecular weights of peptides, observing a greater stimulation of these parameters when biostimulants containing low molecular weight peptides.

It is also important to note that, during the 2017 and 2019 seasons, the mineral content in the olive leaves was lower than in the 2018 and 2020 seasons. This is a consequence of the alternation of the olive trees, which means that a regular harvest is not obtained from one year to the next (Tejada & Benítez, 2020). The foliar application of both biostimulants did not overcome the alternate bearing of the olive trees, although the mineral and photosynthetic pigment contents of the leaves, and consequently the productivity of the trees, improved significantly compared to the control treatment. In this case, differences were also observed in terms of the biostimulant applied, again highlighting the more positive effect of the lower molecular weight of the peptides applied to the plant.

5. Conclusions

Our results suggest that the use of biostimulants from sewage sludge through fermentation processes using *Bacillis licheniformis* is an important step in the valorization of these organic wastes in new energy sources. The foliar application of the biostimulant obtained from sewage sludge after fermentation using the bacterium *Bacillus licheniformis* can be considered a good and sustainable alternative that improves the mineral nutrition of the olive tree, photosynthetic pigment content and, consequently, the olive yield.

This increase was greater when the biostimulant applied had a higher content of low molecular weight peptides, possibly because these peptides are more easily assimilated by the plant.

However, we believe that this study could be the launchpad for future studies in which the greater efficacy of these small molecular weight peptides is corroborated, as well as the doses to be applied and the different application times depending on the vegetative state of the olive tree, different edaphoclimatic conditions and different varieties of olive trees existing in the study area. Similarly, this study could be a starting point for other studies regarding the efficacy of these biostimulants in crops other than olive.

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References

AEMET. (2021). *Agencia Estatal de Meteorología*. Spain. http://www.aemet.es/es/serviciosclimaticos/vigilancia_clima/resumenes?w=1&datos=−1&n=1&k=and

Angin, I., Aslantas, R., Gunes, A., Kose, M., & Ozkan, G. (2017). Effects of sewage sludge amendment on some soil properties, growth, yield and nutrient content of raspberry (*Rubus idaeus* L.). *Erwerbs-Obstbau*, *59*(2), 93–99. https://doi.org/10.1007/s10341-016-0303-9

Ávila-Pozo, P., Parrado, J., Caballero, P., Díaz-López, M., Bastida, F., & Tejada, M. (2021). Use of slaughterhouse sludge in the bioremediation of an oxyfluorfen-polluted soil. *International Journal of Environmental Research*, *15*(4), 723–731. https://doi.org/10.1007/s41742-021-00351-z

Baglieri, A., Cadili, V., Mozzetti Monterumici, C., Gennari, M., Tabasso, S., Montoneri, E., Nardi, S., & Negre, M. (2014). Fertilization of bean plants with tomato plants hydrolysates. Effect on biomass production, chlorophyll content and N assimilation. *Scientia Horticulturae*, *176*, 194–199. https://doi.org/10.1016/j.scienta.2014.07.002

Besnard, G., Terral, J. F., & Cornille, A. (2018). On the origins and domestication of the olive: A review and perspectives. *Annals of Botany*, *121*(3), 385–403. https://doi.org/10.1093/aob/mcx145

Çelik, H., Katkat, A. V., Aşık, B. B., & Turan, M. A. (2010). Effect of foliar-applied humic acid to dry weight and mineral nutrient uptake of maize under calcareous soil conditions. *Communications in Soil Science and Plant Analysis*, *42*(1), 29–38. https://doi.org/10.1080/00103624.2011.528490

Chatzistathis, T., Monokrousos, N., Psoma, P., Tziachris, P., Metaxa, I., Strikos, G., Papadopoulos, F. H., & Papadopoulos, A. H. (2020). How fully productive olive trees (Olea europaea L., cv. 'Chondrolia Chalkidikis') manage to over-satisfy their P nutritional needs under low Olsen P availability in soils? *Scientia Horticulturae*, *265*, 109251. https://doi.org/10.1016/j.scienta.2020.109251

Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R., & Rouphael, Y. (2015). Protein hydrolysates as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 28–38. https://doi.org/10.1016/j.scienta.2015.08.037

Colla, G., & Rouphael, Y. (2015). Biostimulants in agriculture. *Scientia Horticulturae*, *196*, 1–2. https://doi.org/10.1016/j.scienta.2015.10.044

Comission, E. (ed.). (2016). *Circular economy package-proposal for a regulation of the European parliament and of the council* (Vol. 2016/0084).

Duhamel, M., & Vandenkoornhuyse, P. (2013). Sustainable agriculture: Possible trajectories from mutualistic symbiosis and plant neodomestication. *Trends in Plant Science*, *11*(11), 597–600. https://doi.org/10.1016/j.tplants.2013.08.010

Eid, E. M., Shaltout, K. H., Alamri, S. A. M., Alrumman, S. A., Hussain, A. A., Sewelam, N., El-Bebany, A. F., Alfarhan, A. H., Picó, Y., & Barcelo, D. (2021). Prediction models based on soil properties for evaluating the uptake of eight heavy metals by tomato plant (Lycopersicon esculentum Mill.) grown in agricultural soils amended with sewage sludge. *Journal of Environmental Chemical Engineering*, *9*(5), 105977. https://doi.org/10.1016/j.jece.2021.105977

El-Sanatawy, A. M., AshShormillesy, S. M. A. I., El-Yazied, A. A., El-Gawad, H. G. A., Azab, E., Gobouri, A. A., Sitohy, M., & Osman, A. (2021). Enhancing grain yield and nitrogen accumulation in wheat plants grown under a Mediterranean arid environment by foliar spray with papain-released whey peptides. *Agronomy*, *11*(10), 1913. https://doi.org/10.3390/agronomy11101913

Kandil, A. A., Sharief, A. E. M., Seadh, S. E., & Altai, D. S. K. (2016). Role of humic acid and amino acids in limiting loss of nitrogen fertilizer and increasing productivity of some wheat cultivars grown under newly reclaimed sandy soil. *International Journal of Advanced Research in Biological Science*, *3*(4), 123–136. http://s-o-i.org/1.15/ijarbs-2016-3-4-18

Kapoore, R. V., Wodd, E. E., & Llewellyn, C. A. (2021). Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. *Biotechnology Advances*, *49*, 107754. https://doi.org/10.1016/j.biotechadv.2021.107754

Kocira, A., Lamorska, J., Kornas, R., Nowosad, N., Tomaszewska, M., Leszczyńska, D., Kozłowicz, K., & Sylwester Tabor, S. (2020). Changes in biochemistry and yield in response to biostimulants applied in bean (Phaseolus vulgaris L.). *Agronomy*, *10*(2), 189. https://doi.org/10.3390/agronomy10020189

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthesis biomembranes. *Methods in Enzymology*, *148*, 350–383. https://doi.org/10.1016/0076-6879(87)48036-1

MAPA, (1986). *Métodos oficiales de análisis*. Ministerio de Agricultura, Pesca y Alimentación 1. pp. 221–285.

Onofrei, V., Teliban, G. C., Burducea, M., Lobiuc, A., Sandu, C. B., Tocai, M., & Robu, T. (2017). Organic foliar fertilization increases polyphenol content of Calendula officinalis L. *Industrial Crops and Products*, *109*, 509–513. https://doi.org/10.1016/j.indcrop.2017.08.055

Quartieri, M., Cavani, L., Lucchi, A., Marangoni, B., & Tagliavini, M. (2022). Effects of the rate of protein hydrolysis spray concentration on growth of potted kiwifruit (Actinidia deliciosa) plants. *Acta Horticulturae*, *594*(594), 341–347. https://doi.org/10.17660/ActaHortic.2002.594.42

Radkowski, A., & Radkowska, I. (2018). Influence of foliar fertilization with amino acid preparations on morphological traits and seed yield of timothy. *Plant, Soil and Environment*, *64*(No. 5), 209–213. https://doi.org/10.17221/112/2018-PSE

Rodríguez-Morgado, B., Caballero, P., Paneque, P., Gómez, I., Parrado, J., & Tejada, M. (2019). Obtaining edaphic biostimulants/biofertilizers from sewage sludge using fermentative processes. Short-time effects on soil biochemical properties. *Environmental Technology*, *40*(3), 399–406. https://doi.org/10.1080/09593330.2017.1393016

Rodríguez-Morgado, B., Gómez, I., Parrado, J., García-Martínez, A. M., Aragón, C., & Tejada, M. (2015). Obtaining edaphic biostimulants/biofertilizers from different sewage sludges. effects on soil biological properties. *Environmental Technology*, *36*(17), 2217–2226. https://doi.org/10.1080/09593330.2015.1024760

Sarijeva, G., Knapp, M., & Lichtenthaler, H. K. (2007). Differences in photosynthetic activity, chlorophyll and carotenoid levels, and in chlorophyll fluorescence parameters in green sun and shade leaves of Ginkgo and Fagus. *Journal of Plant Physiology*, *164*(7), 950–955. https://doi.org/10.1016/j.jplph.2006.09.002

Searchinger, T. (2013). *The great balancing cct: Installment 1 of creating a sustainable food future*. World Resources Institute.

Tejada, M., & Benítez, C. (2020). Effects of different organic wastes on soil biochemical properties and yield in an olive grove. *Applied Soil Ecology*, *146*, 103371. https://doi.org/10.1016/j.apsoil.2019.103371

Tejada, M., & Gonzalez, J. L. (2003). Influence of foliar fertilization with amino acids and humic acids on productivity and quality of asparagus. *Biological Agriculture & Horticulture*, *21*(3), 277–291. https://doi.org/10.1080/01448765.2003.9755270

Tejada, M., Rodríguez-Morgado, B., Gómez, I., Franco-Andreu, L., Benítez, C., & Parrado, J. (2016). Use of biofertilizers obtained from sewage sludges on maize yield. *European Journal of Agronomy*, *78*, 13–19. https://doi.org/10.1016/j.eja.2016.04.014

Tejada, M., Rodríguez-Morgado, B., Paneque, P., & Parrado, J. (2018). Effects of foliar fertilization of a biostimulant obtained from chicken feathers on maize yield. *European Journal of Agronomy*, *96*, 54–59. https://doi.org/10.1016/j.eja.2018.03.003

WRB. (2014). World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. *Food and Agriculture Organization of the United Nations: Roma, 192*. https://www.fao.org/3/i3794en/I3794en.pdf

Ye, L., Zhao, X., Bao, E., Li, J., Zou, Z., & Cao, K. (2020). Bio-organic fertilizer with reduced rates of chemical fertilization improves soil fertility and enhances tomato yield and quality. *Scientific Reports*, *10*(1), 177. https://doi.org/10.1038/s41598-019-56954-2

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