agriculture

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Zeolite and *Ascophyllum nodosum*-Based Biostimulant Effects on Spinach Gas Exchange and Growth

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Abstract: Among the innovative practices of dry-farming in recent years, the possibility of the combined use of biostimulants and soil conditioners is assuming an important role. In a preliminary pot experiment, this study aimed to verify the combined effects of *Ascophyllum nodosum*-based biostimulant and zeolite applied to the soil on gas-exchange and spinach growth. We also monitored the soil water content to study the effect on spinach soil water uptake. Pots were filled with soil to which zeolite and an *Ascophyllum nodosum*-based biostimulant were added. Spinach plants grew into pots and were subjected to four treatments: (1) soil plus zeolite at a percentage of 1%, (2) soil plus the biostimulant, (3) soil plus zeolite at 1% and biostimulant, (4) bare soil as control. The use of the zeolite and the *A. nodosum*-based biostimulant led to a higher (+10%) soil water content, highlighting the positive role in allowing a good water uptake by the spinach plant. Plant growth was not changed, while only photosynthesis showed an increase equal to 6% in spinach plants. These results are discussed with the soil water content variation according to modification induced by treatments. The combined use of zeolite and *A. nodosum*-based biostimulant can be considered a strategy to improve water storage and, at the same time, improve spinach cultivation in terms of sustainability.

Keywords: soil moisture; available water content; soil conditioner; *Spinacia oleracea* L.

Citation: Castronuovo, D.; Comegna, A.; Belviso, C.; Satriani, A.; Lovelli, S. Zeolite and *Ascophyllum nodosum*-Based Biostimulant Effects on Spinach Gas Exchange and Growth. *Agriculture* **2023**, *13*, 754. https://doi.org/10.3390/agriculture13040754

Academic Editors: Vladimír Frišták and Martin Pipíška

Received: 7 March 2023
Revised: 19 March 2023
Accepted: 22 March 2023
Published: 24 March 2023

1. Introduction

In the current climate change scenarios, finding new solutions to support agricultural crop yield in hot arid climate environments, such as the Mediterranean, becomes crucial [1,2]. In arid and semiarid-environments, water management in dry crop systems is essential to ensure the water needed for food production and to develop resilience to deal with future risks and uncertainties related to water [3,4]. Soil water content is an essential component of the surface water budget, determining cropping systems' health or stress, such as those in agriculture and agroforest [5,6].

Among the innovative practices of dry-farming in recent years, the possibility of the combined use of biostimulants and soil conditioners is assuming an important role [7–10]. The knowledge and know-how of these innovative amendments must be improved to use them as a valid alternative to non-natural molecules to obtain a higher crop yield and decrease the undesirable effects on the environment of their use in agriculture [10]. Biostimulants are increasingly being used to improve crop growth and water use, while soil amendments are to improve water holding capacity. Among the many biostimulants currently on the market, those containing *A. nodosum* are presently considered the most interesting and most popular due to their numerous effects on some crops [10,11]. Seaweed extracts obtained by *A. nodosum* contain chelators [12] and, between them, alginates which contribute to the soil aeration and water-holding capacity [13,14]. Due to the little-known mechanism of these molecules and the different formulations of *A. nodosum*-based biostimulants on the market, the outcome of using these biostimulants on crops in the open field is disputed [15,16].

Among the various types of soil conditioners, zeolites, which are microporous crystalline aluminosilicate minerals, are considered amendments because they can improve soils' ability to retain water and their cation exchange capacity [17,18]. Nakhly et al. summarized in their review the positive effect of zeolite on soil nutrient and water retention, highlighting the contrasting effects of these aluminosilicates concerning their heterogeneous origin and composition [19]. Zeolites synthesized from coal fly ash (FA), a waste product of burning coal in thermo-electric power plants, have also been studied for their effects on sunflower growth at different soil concentrations [18]. Two conflicting effects were observed during the field application of zeolite: high water retention capacity and low hydraulic conductivity, according to the soil texture and amount of zeolite added [18,20,21].

Both the use of biostimulants and zeolite-based soil amendments represent an innovative but, at the same time, very new field of investigation. In particular, there needs to be more information on the combined effect of these materials on crops [9], even if they have been used frequently by farmers in recent years.

Among the various vegetable crops, spinach (*Spinacia oleracea* L.), because of its nutritional properties, availability, and use both as fresh vegetable and frozen food, is an economically important leafy crop in many countries [22]. In fact, the world's spinach production is 32,294,452 tons, of which 30,855,894 and 775,476 tons are produced in Asia and Europe, respectively [23].

This study aimed to verify the combined effects of *A. nodosum*-based biostimulant and zeolite on gas exchange and growth of spinach. We also monitored the soil water content to study the effect on spinach soil water uptake.

2. Materials and Methods

2.1. Experimental Description and Experimental Design

To assess the effect of coal fly ash zeolite and an *Ascophyllum nodosum*-based biostimulant on spinach (*Spinacia oleracea* L. cv 'Lorelay') growth and yield, an experiment was conducted in controlled conditions at the Agronomy Laboratory of the University of Basilicata. Polypropylene plastic pots having a conical trunk shape (20.0 cm high, 17.0 cm lower diameter, 20.0 cm upper diameter) with a volume of 5 L were used to cultivate the spinach plants. Pots were filled with soil, collected in a field located in the Basilicata Region Southern Italy (40°33′31″ N, 15°45′31″ E; 800 m a.s.l.), air-dried, and passed through a 2 mm sieve to which zeolite and an *Ascophyllum nodosum*-based biostimulant (Acadian MPE, Acadian Seaplants Limited, Dartmouth, Canada) were added in order to have four experimental treatments: (1) soil plus zeolite at a percentage of 1% (Zeo); (2) soil plus the biostimulant (Bios); (3) soil plus zeolite 1% and biostimulant (Zeo + Bios); (4) bare soil as control (Table 1).

Table 1. Main physicochemical properties of the investigated soil.

Property	Soil	Unit	Method
Sand	9.53	%	Hydrometer method
Silt	66.18	%	
Clay	24.29	%	
Texture (USDA classification)	Silty loam	-	
Soil bulk density (ρ_b)	1.369	g/cm^3	Core method
Organic matter	34.90	g kg^{-1}	Walkley–Black
Cation exchange capacity	27.85	cmol/kg	$BaCl_2$ pH 8.1
pH (in H_2O 1:2.5)	7.63		pH meter
Wilting point (WP)	25.5	% vol	Retention curve (at h = −1.5 MPa)
Field capacity (FC)	40.5	% vol	Retention curve (at h = −0.03 MPa)

A randomized block design with four treatments and three replicates was carried out to have a total of twelve pots. Both soil and zeolite used in the trial were previously characterized by Belviso et al. [18], as shown in Table 1. On 28 April 2022, the pots were irrigated to the field capacity (Table 1), and then spinach seeds were sowed to have 2 plants per pot.

On 19th May, fertilization with potassium nitrate 13–46 with a dose of 1 g of N plant^{-1} and 3.7 g of K_2O plant^{-1} was made. Then, on the 1st and 13th of June, the Acadian biostimulant was added to the soil with irrigation water, considering a dose equal to 2.5 g L^{-1}. During the experiment, plants were watered to ensure good soil water content in pots, slightly above field capacity (Table 1), and soil moisture content was monitored continuously by soil sensors during the trial. In fact, at the beginning of the trial, four watermark probes (Model Watermark 200SS, The Irrometer Company, Inc., Riversice, CA, USA) were installed in representative pots (one watermark per treatment). Before the trial, according to the installation and operating instructions, watermark probes (Watermarks 200SS, Irrometer Company Ltd., Riverside, CA, USA) underwent preconditioning [24], and then they were calibrated according to the methodology proposed by Abbas et al. [25]. Moreover, using a SPAD meter (SPAD-502, Konica Minolta Corporation Ltd., Tokyo, Japan), the leaf greenness index values were measured. Leaf water potential (Ψ) was measured on the youngest uppermost fully expanded leaf of three plants per treatment at midday, using the pressure chamber technique (Scholander pressure chamber), according to Scholander et al. [26]. Gas exchange parameters (photosynthesis, transpiration, stomatal conductance, and intercellular CO_2 concentration) were measured using an LI-6400 portable photosynthesis system equipped with a 2 cm^2 chamber and 6400-40 LED light source (LI-COR Inc., Lincoln, NE, USA) operating at 380 ppm ambient CO_2 concentration. Measurements were conducted between 12:00 and 14:00 h (solar time) under saturating light conditions (active photosynthetic radiation, PAR approximately 1500 μmol photons m^{-2} s^{-1}). Water Use Efficiency (WUE) was calculated as the ratio between assimilated CO_2 and transpiration flow [27]. On the 28th of June, at the end of the trial, per each pot, leaves were counted and passed through a surface electronic detector (Model 3100, LI-Cor, Inc., Lincoln, NE, USA) to measure leaf area. Lastly, dry weight, by drying the samples in a ventilated oven at 75 °C until a constant weight was reached, and humidity (%) were determined.

2.2. Statistical Analysis

Before performing analysis of variance (ANOVA), Shapiro–Wilk ($p \leq 0.05$) and Bartlett ($p \leq 0.05$) tests were applied to test normality and homogeneity of variances, respectively. Afterwards, data were subjected to the analysis of variance (ANOVA), considering the "soil mixtures" as a source of variation. Mean values were separated with the Least Significant Difference (LSD) test, at the significance level of $p \leq 0.05$.

All statistical procedures were computed using the using the software RStudio: Integrated Development for R, version 2022.12.0+353 [28].

3. Results

Figure 1 shows soil water volumetric content variation in the pots of treatments compared during the duration of the experiment. As can be observed only in the second part of the experiment, the soil water content differs in the compared treatments. At the end of the experiment, a higher soil water content was observed in the treatment with 1% zeolite (Zeo), equal to 33.21%. A lower water content of 30.2% was observed in the Bios and Zeo+Bios treatments. Finally, the lowest value was measured in control, equal to 27.3%. At the end of the test, while remaining within the range of available water content, the soil water content was never dropped below the wilting point (25.5%); in fact, soil water content equaled 30.2% both in the Bios treatment and in the Zeo + Bios treatment, which highlights the positive role of the biostimulant in allowing a good water uptake by the spinach plants, given that at 30.2% of soil water content, the soil matrix potential is less negative than that corresponding to the 33.2%, measured in the Zeo treatment. According to these results, we observed the least negative value of leaf water potential and, consequently, better water status of spinach plants in the treatment with *A. nodosum* biostimulant (Bios), equal to −1.90 MPa (Table 2).

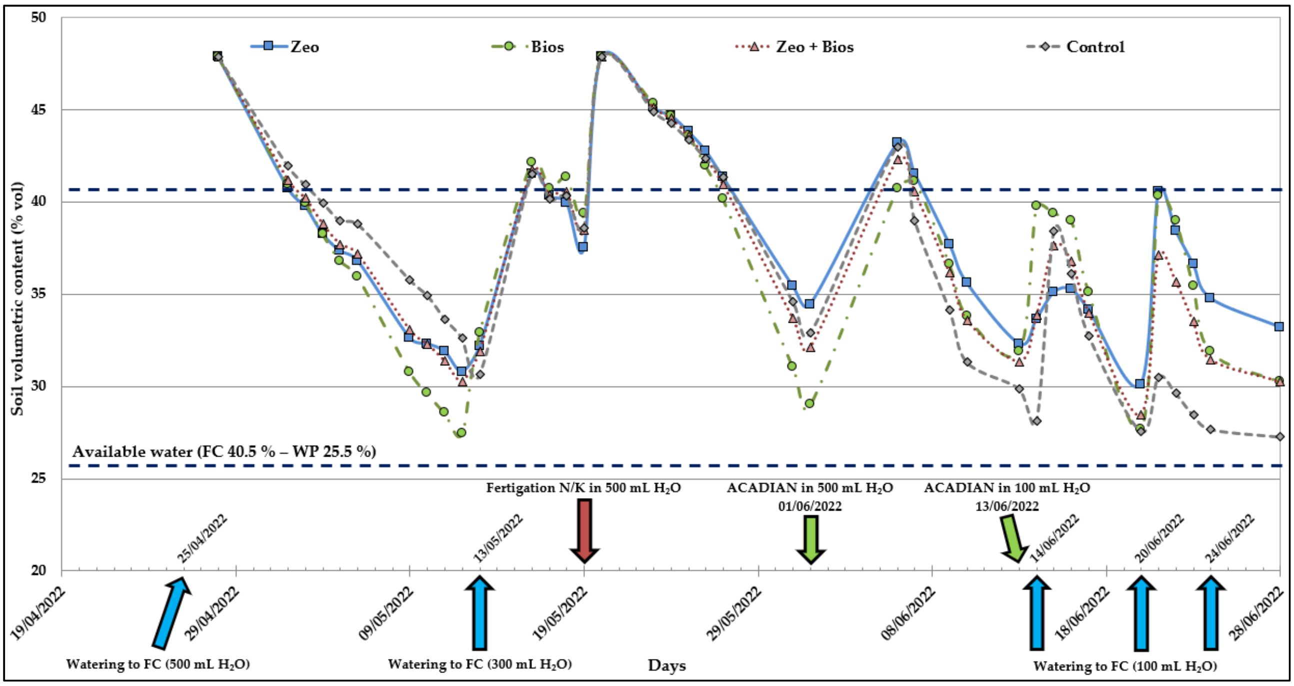

Figure 1. Soil water content trend measured by watermark probes during spinach growing cycle treated with coal fly ash zeolite (Zeo), *A. nodosum*-based biostimulant (Bios), and zeolite plus *A. nodosum*-based biostimulant (Zeo + Bios). Values are mean (n = 3).

Table 2. Effects of treatments on some morphological and physiological traits of spinach and on soil volumetric water content.

Treatments (1)	Leaf Number	Leaf Area	Leaf Fresh Weight	Leaf Dry Weight	Dry Matter Content	Leaf Water Potential (Ψ)	SPAD	Soil Volumetric Water Content (2)
	(n.)	(cm^2)	(g)	(g)	(%)	(MPa)		(%)
Zeolite	7 b	44.60	1.58	0.22	14.10 b	−1.97 bc	53.33	33.21 a
Biostimulant	8 a	43.80	2.19	0.29	13.51 b	−1.90 c	50.90	30.25 ab
Zeo + Bios (3)	7 b	48.42	1.54	0.22	14.11 b	−2.05 ab	52.13	30.24 ab
Control	7 b	44.83	1.68	0.27	16.16 a	−2.17 a	54.20	27.26 b
Significance(4)	*	ns	ns	ns	*	*	ns	*

(1) Mean values followed by a different letter are significantly different at $p \leq 0.05$, according to LSD test. (2) Data recorded on 28 June 2022. (3) Zeolite plus *A. nodosum*-based biostimulant (Zeo + Bios). (4) ns, no significant difference; *, Significance at $p \leq 0.05$.

Regarding the effect on the gas exchange parameters of the spinach leaves, we observed a higher photosynthetic activity in the Bios treatment, equal to 13.53 µmolCO_2 m^{-2}s^{-1} but no effect on stomatal conductance. Leaf transpiration was higher with Bios + Zeo treatment, equal to 2.85 mmolH_2Om^{-2}s^{-1}, but non-different to Bios treatment (2.06 mmolH_2Om^{-2}s^{-1}) (Figure 2). These transpiration values determined, as expected, a greater WUE on Bios treatment, equal to 6.94 µmolCO_2 molH_2O^{-1} (Figure 2). While, the Zeo + Bios combined treatment did not differ from the other treatments (Figure 2). These results disagree with other authors [29,30]; however, few studies report the effect of seaweed biostimulant obtained from *A. nodosum* on spinach gas exchanges. Under full irrigation on spinach, there would seem to be no effect on leaf gas exchange, while under water stress conditions, a positive effect on photosynthesis has been demonstrated [8]. The growth of the spinach plants was not significantly influenced by the different compared treatments; however, the number of leaves was higher in the Bios treatment, equal to 8 (Table 2). Indeed, leaf area, fresh leaf weight, and SPAD did not vary significantly (Table 2).

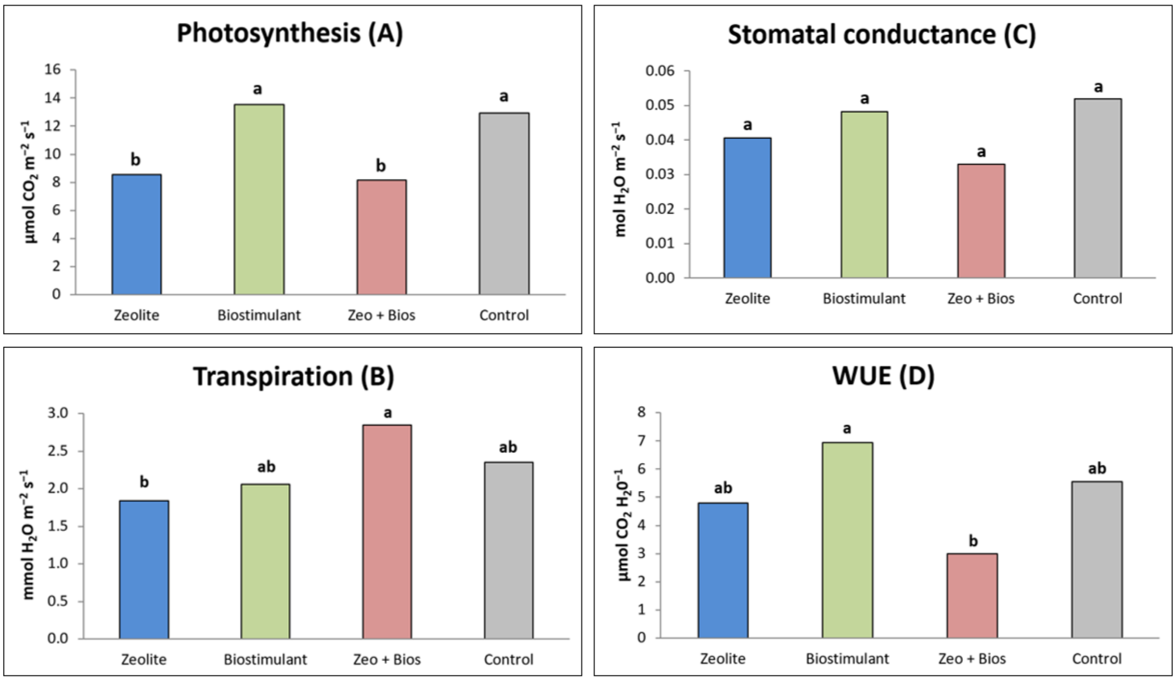

Figure 2. Photosynthetic rate (**A**), stomatal conductance (**B**), transpiration rate (**C**), and water use efficiency (**D**) measured at the end of the experiment in leaves of spinach plants treated with zeolite, *A. nodosum*-based biostimulant, and zeolite plus *A. nodosum*-based biostimulant (Zeo + Bios). Mean values (n = 4) within a column followed by different lowercase and uppercase letters are significantly different at $p < 0.05$, respectively, according to the Least Significant Difference test.

4. Discussion

Other authors showed no effect of *A. nodosum* biostimulant on leaf water content under full irrigation [8]. Although several studies have highlighted the positive effect of seaweed extracts on plant growth and water relations, other studies have given conflicting results [31,32]. Furthermore, the different effects observed can also be induced by the different forms of application of the biostimulant, which can be foliar or directly to the soil [30,33]. Our results also show the positive, although slight, combined effect of zeolite and *A. nodosum*-based biostimulant on soil water holding capacity. We showed in previous research the strong water-holding capacity of the zeolite, especially in silty-loam soil; furthermore, hydraulic conductivity at saturation (Ks) already decreased by 20% with 1% of zeolite [18]. This effect, which is undoubtedly desired in arid environments regarding soil water storage capacity, can nevertheless weaken the plant water uptake from the soil, since the zeolite, especially at high concentrations, causes a change in the soil's porosity and aggregates [18]. Consequently, water, as the effect of porosity reduction, is less available for plants because the soil water potential becomes more negative as the pore radius decreases [18,34]. In this way, the combined use of zeolite with the biostimulant in silty-loam texture soils can mitigate the effect of reducing porosity and enhance its beneficial effects. Our results on spinach gas exchange parameters agree with other authors [8]. Generally, seaweed biostimulants obtained from *A. nodosum* could promote growth and nutritional quality and mitigate drought stress by improving phenolic and antioxidant content [11]. Nevertheless, the slight and insignificant effect on some parameters is again due to the variability induced by the type of treatment (foliar or soil application) [35] and soil texture [36]. Seaweed extracts have two kinds of action when applied to the soil, as in our case, they promote the growth and yield capacity of the crop and, since they are also chelators, can contribute directly to soil health [12]. This mechanism is due to the alginic acids, the principal constituent of the algal cell wall. In this way, when applied to the soil through natural chelation, seaweed extracts may absorb water, improving soil aeration and water storage [37]. For this reason, the effect of this biostimulant on spinach, when applied directly to the soil, may vary according to the soil texture, as shown by other authors on leaf number, area, and stem diameter of broccoli crops [36]. The effects observed in our experiment become even more difficult to explain when, in addition to applying the biostimulant to the soil, a soil conditioner such as zeolite is applied, since zeolite's effect on soil hydrological proprieties also depends on soil texture, as reported in a previous paper [18] and by Garboswski et al. [10] in their review. As a general indication, mineral amendments, such as zeolite, usually lead to an increase in soil compactness and therefore should be used with great caution in very clayey soils or loamy soils; in these soils, it is better to add algae-based amendments or plant residues to reduce the formation of a soil crust [10].

One of the main requests of modern agriculture is to find production systems that allow obtaining good quality food without depleting environmental resources. This demand can be met through the use of biofertilizers. Natural amendments, and therefore also seaweed extracts or zeolites, can be a valid aid in pursuing the sustainability of cropping systems [10].

5. Conclusions

The combined use of zeolite and *A. nodosum*-based biostimulant can be considered a strategy to improve water storage and improve spinach cultivation with a sustainable approach. Using the biostimulant and zeolite resulted in a change in the soil water content, quantified by a 10% increase, highlighting the positive role of allowing good water uptake equal to 6% by the spinach plant for the soil texture considered in this trial (silty loam soil). Further research should be planned to elucidate the effect on spinach and whether the effect of a soil conditioner such as zeolite and the use of a seaweed biostimulant obtained from *A. nodosum* also concern the discordant effects observed about the soil texture. The results obtained in this preliminary experiment highlight the need to investigate in more depth the effects that the combined use of soil conditioners and biostimulants can determine on spinach crops before the use of both these tools becomes more widespread.

Author Contributions: Conceptualization, D.C. and S.L.; methodology, D.C., S.L., A.C. and C.B.; software, D.C., S.L. and A.C.; validation, D.C., S.L., A.C. and A.S.; formal analysis, D.C. and S.L.; investigation, D.C. and S.L.; resources, S.L.; data curation, D.C. and S.L.; writing—original draft preparation, D.C. and S.L.; writing—review and editing, D.C., S.L., A.C. and A.S.; visualization, D.C. and S.L.; supervision, D.C. and S.L.; project administration, S.L.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

Acknowledgments: We thank Giuseppe Mercurio for his technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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