



Article Biostimulants for Resilient Agriculture: A Preliminary Assessment in Italy

Rita Leogrande ¹, Daniel El Chami ^{2,*}, Giulio Fumarola ³, Michele Di Carolo ³, Giuseppe Piegari ², Mario Elefante ², Donato Perrelli ³ and Crescenza Dongiovanni ³

- ¹ Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Via C. Ulpiani 5, I-70125 Bari, Italy; rita.leogrande@crea.gov.it
- ² TIMAC AGRO Italia S.p.A., S.P.13, Località Ca' Nova, I-26010 Ripalta Arpina, Italy; giuseppe.piegari@roullier.com (G.P.); mario.elefante@roullier.com (M.E.)
- ³ Centre for Research, Experimentation and Training in Agriculture Basile Caramia (CRSFA), I-70010 Locorotondo, Italy; giuliofumarola@crsfa.it (G.F.); micheledicarolo@crsfa.it (M.D.C.); donatoperrelli@crsfa.it (D.P.); enzadongiovanni@crsfa.it (C.D.)
- * Correspondence: daniel.elchami@roullier.com; Tel.: +39-0373-669-233; Fax: +39-0373-669-291

Abstract: In agriculture, plant biostimulants have become necessary to meet the United Nations sustainable development goals (UN-SDGs) and advance the European Green Deal. In particular, seaweed-based biostimulants have received a greater acceptance for their several benefits in crop growth and yield. In this study, we evaluated the effects of foliar applications of a vegetable- and brown-algae-based extract (*Ascophyllum nodosum* (L.) Le Jol. on grapes (*Vitis vinifera* L. cv. Montepulciano) and olives (*Olea europaea* L. cv. Coratina)) and its agronomic performance in two field experiments in the Apulia region, which is known for its modern agricultural sector. The results highlight that the crop responses differ in grape and olive orchards. The biostimulant application determined significant increases in bunch development (+9.5%) and bunch weight (+10%) compared to the untreated control. In the olive orchard, the yield was not significantly influenced by biostimulant application, whereas we observed quality improvement in the olive oil of the treated plants compared to the control. To better understand the mechanisms behind this difference, the research concludes by suggesting that further research pursues in-depth studies and high scientific and technical proficiency to determine and optimise the rates and timing of applications.

Keywords: biostimulants; vegetable extract; seaweed extract; agrosystems; resilience; Apulia (Italy)

1. Introduction

The two-way relationship between agriculture and climate change poses a serious constraint on reaching food security and availability [1]. The action plan for sustainable development, which began in 1992 with Agenda 21 and continued in 2015 with Agenda 2030, set specific objectives for this transformative development [2]. As a response, the European Union launched the "European Green Deal" in 2020 to sustainably develop the member states' economies without increasing resource deterioration (https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en, 22 February 2022). The agricultural strategy of the deal, the Farm to Fork strategy (F2F), established different targets to adapt European agriculture to climate change and increase its resilience [3]. Furthermore, the main targets of the F2F strategy include the reduction of fertilisers by 20% and nutrient losses by at least 50% [3] by adopting different sustainable practices for crop production [4].

Within this context, the available scientific evidence has documented the importance of organic and mineral nutrition for overall sustainable development [5], in particular, for crop growth and health [6], soil fertility [7,8], and productivity [9,10]. However, reaching the peak of crop nutrient uptake, the new paradigm for sustainable agriculture, based on



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). the integration of scientific and technological advances in nutrition, puts soil, health, and the environment at the centre to efficiently manage highly productive agrosystems [4,11].

For this purpose, biostimulants represent a promising field in crop nutrition, are fundamental to reaching sustainable agrosystems, and are resilient against climate change [4,12,13]. Biostimulants were first defined by Zhang and Schmidt [14]. More recently, Du Jardin [15] described them as "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield".

The literature has identified different categories of biostimulants, which still need thorough assessment in agriculture to answer all aspects of their use under different conditions and crops [15]. Seaweed extracts and botanicals represent an important biostimulant category, possessing plant-growth-promoting activities that still need a thorough assessment to transform this traditional knowledge into scientific evidence [4,16–18]. The extracted substances act on plants and soils, and their action is intensively studied to understand all the physical, chemical, and biological aspects that control it and assess its effects.

Indeed, a handful of studies investigated the effects of seaweed and botanical extracts on plant metabolism and physiological health [19,20] and their phytohormone-like activity [21]. More recently, the research involved gene expression analysis, highlighting the effects of seaweed and botanical extracts on crops' metabolic regulatory pathways [22]. Others assessed the changes in soil properties (biological, physical, and biochemical) and their relation to nutrients' uptake efficiency [23–25]. Furthermore, seaweed and botanical extracts enhance the performance of plants under abiotic stresses, such as tolerance to freezing temperatures and high temperatures [26,27], drought, water, and salinity [28,29]. Agronomic efficiency has also been subject to different assessments to evaluate seaweed and botanical extracts' effects on improving productivity, product quality, and shelf life [13,30,31].

However, conflicting statements were reported on the effects of biostimulant applications in different climatic conditions occurring year after year in open-field cultivation systems. Indeed, Soppelsa et al. [32] observed positive effects of biostimulant application on yield performance in an apple orchard for two consecutive years, highlighting that the results obtained were independent of the impact of the seasonal climatic conditions. Similarly, Frioni et al. [33] showed beneficial effects of the seaweed extract foliar applications on fruit quality for several red grapevine cultivars over various climatic conditions (from cold to warm viticultural regions). In contrast, other studies noted different behaviours of the crops treated with biostimulants due to different climatic conditions [34,35]. In particular, Gutiérrez-Gamboa et al. [34] observed that seaweed applications on grapevines had a differential effect on must amino acids content, which depended on the climate conditions of the season. The authors found that during the driest season, the low dosage of the seaweed applications increased the concentration of several amino acids in musts, while in the rainy season, the concentration of certain amino acids in musts increased only with a high biostimulant dosage.

Furthermore, Chanda et al. [36] stated that not all microalgae species have significant biostimulant effects on plant growth, suggesting that the biostimulant properties are due to "species-specific" metabolites produced by particular microalgae species.

However, acknowledging that climate change effects vary in time, space, and intensity [37], adaptation in agriculture is related to sustainable agriculture and should be case-specific, and needs to be assessed and evaluated, considering specific field conditions [38]. Therefore, unlike previous studies, this research aimed to evaluate the agronomic and organoleptic effects of seaweed and botanical extract produced at the CMI Roullier laboratories (Centre Mondial de l'Innovation Roullier). Thus, we assessed the qualitative and quantitative performance of different management practices of two strategic crops in the Apulia region's (Italy) agriculture, using field experiments and satellite imaging.

2. Material & Methods

The experimental fields are located in Brindisi province in the Italian Apulia region, known for its modern agricultural sector. In particular, Apulia is placed first among the grape-growing areas of Italy and has 35.5% of the total olive area (Table 1). The climate is typically Mediterranean, with mild winters and hot summers. Figure 1 shows the average weather data collected between 1999 and 2019, with monthly average variations.

Table 1. Agricultural area of grapes and olives.

Indicator	Italy	Apulia
Average Farm Size (ha)	11.0	6.6
Total Agricultural Area (ha)	12,598,161	1,285,274
Grape Agricultural Area (ha) [†]	614,958	92,038
Olive Agricultural Area (ha) [‡]	1,032,858	366,897

⁺ Grape agricultural area includes grapevine and table grape varieties. [‡] Olive agricultural area has table and oil olive varieties. Source: Istat [39] License: Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).



Figure 1. Average annual and monthly precipitation (mm) and temperature (°C) between 1999 and 2019 in Brindisi.

2.1. Experimental Design

The field trials were performed in *Olea europea* L. and *Vitis vinifera* L. orchards in Ostuni and Carovigno in Brindisi province (Figure 2). In both cases, the field trial was divided into four experimental plots, each of 2000 m², two of which were treated with vegetables and brown algae extract (*Ascophyllum nodosum* (L.) Le Jol.) in foliar spray, with the other two left untreated (Figure 2). The product was formulated in the CMI Roullier laboratories (Centre Mondial de l'Innovation Roullier) [19,22] to act on the following physiological aspects:

- Improve the photosynthetic activity of the crop and biomass development;
- Ameliorate resistance to biotic and abiotic stressors;
- Improve the overall crop nutrition.



Figure 2. Maps of the case study within the regional and local context.

2.1.1. First Experiment

The trial was carried out in 50 year-old olive orchards in loamy soil at 30 m above sea level (Table 2). The orchard consisted of Coratina and Leccino cultivars, spaced at 10.0×4.0 m, and planting density was around 250 plants ha⁻¹. Two plots of 2000 m² were selected for each treatment within the experimental field.

Table 2. Infographics of the case study.

	Experiment 1: Olive Orchard	Experiment 2: Grape Orchard
Location	Carovigno	Ostuni
Coordinates	40°43′33.8″ N 17°43′30.5″ E	40°42′29.8″ N 17°23′27.2″ E
Variety	Leccino	Montepulciano
Age	50	31
Soil Type	Loam	Clay Loam
Soil pH	7.9	7.3
EC (μ S cm ⁻¹)	0.63	0.14
% Sand (2–0.05 mm)	36.0	37.5
% Lime (0.05–0.002 mm)	38.3	31.7
% Clay (<0.002 mm)	25.7	30.8
Total Calcium (g/kg)	20	16

Agricultural practices were similar for all plots in fertilisation, irrigation, and phytosanitary applications. Treated plots in the olive orchard received two foliar applications (3 L ha⁻¹ each), first at veraison and the second twenty days after. Applications were performed with a 1000 L atomiser equipped with six nozzles per side, each at 2.5 mm in diameter, delivering a water mixture volume of 1.500 L ha⁻¹.

2.1.2. Second Experiment

The experimental design of the grape orchard was set up in clay loam soil at 302 m above sea level on Montepulciano cultivar planted in 1991 in a tendon training system (Table 2). Plants spacing between rows and within the row was $2.5 \text{ m} \times 2.5 \text{ m}$ with an eastwest orientation and planting density of around 1600 plants ha⁻¹. Two plots of 2000 m² were selected for each treatment within the experimental field. Within each plot two subplots, consisting of 10 plants each, were identified and all subsequent investigations were carried out on these selected plants.

The agronomic practices and phytosanitary application performed in the orchard were the same for all plots. The biostimulant was applied three times at a 3 L ha⁻¹ each time, using an atomiser equipped with ten nozzles each with 1.5 mm cone diameters and a distribution volume equivalent to 1000 L ha⁻¹. The first application was at 10 cm shoot length, the second at the pre-flowering stage, and the third at the post-fruit set.

2.2. Data Collection

2.2.1. First Experiment

In each plot of the olive orchard, we selected ten plants of Leccino cultivar for data collection, for a total of 20 plants per treatment. We determined the yield at harvest (first 10 days of November) and extracted oil from representative fruits of each plot (10 kg).

Free fatty acids (acidity), number of peroxides, and spectrophotometric examination in the ultraviolet (specific extinction at 232 nm (K232), specific extinction at 270 nm (K270), and the variation of specific extinction (Δ K)), were carried out in accordance with official European method of olive oil analysis (Reg. CEE 2568/1991 11/07/1991 GU CEE L248 05/09/1991 and subsequent modifications).

The total polyphenol content in the olive oil was determined by a colorimetric reaction with the Folin–Ciocalteau reagent. Briefly, 10 mL of a methanol–water (80:20) mixture was added to 10 g of olive oil. After shaking and centrifugation, two phases were obtained and the upper phase was recovered one more time to extract all residual polyphenols in the remaining oil. Then, 1 mL of extract, 1 mL of Folin–Ciocalteau reagent (Titolchimica, Pontecchio Polesine (RO), Italy), and 9 mL of 7.5% sodium carbonate (Sigma-Aldrich, Saint Louis, MO, USA) solution were introduced into a test tube. At the same time, a blank was prepared with 1 mL of Folin–Ciocalteau reagent, 9 mL of 7.5% sodium carbonate solution, and 1 mL of methanol–water mixture. After two hours, measures were carried out at 765 nm using a UV-Vis spectrophotometer Evolution 201 (Thermo Scientific, Waltham, MA, USA). The results were expressed in gallic acid by constructing a calibration curve in the range of 25–500 mg L⁻¹.

2.2.2. Second Experiment

In each plot of the grape orchard, we identified two subplots, each with 10 plants, to constitute four replicates per treatment, for a total of 40 plants per treatment for data collection.

For the selected plants, we measured the rachis length on all bunches three times during the experiment: at 10 cm shoot length (before biostimulant application), at the pre-flowering stage, and at the post-fruit set. In addition, from veraison to harvesting time, four samplings of 1 kg each were randomly performed, taking portions of bunches (3–4 grapes) from each subplot for each plant at different exposures and positions.

The grape juice obtained from each sample (4 samples per treatment) was filtered and subjected to multiparametric analysis through the FT-IR chemometric technique using the WineScan Flex instrument (FOSS, Hilleroed, Denmark). The following parameters were determined: reducing sugars (g L⁻¹); glucose (g L⁻¹); fructose (g L⁻¹); sugar degree (°Bx); pH, total acidity (g L⁻¹), volatile acidity (g L⁻¹); total dry extract (g L⁻¹); malic acid (g L⁻¹); tartaric acid (g L⁻¹); gluconic acid (g L⁻¹); citric acid (g L⁻¹); potassium (g L⁻¹); absorbance at 420 nm (A420), 520 nm (A520), 620 nm (A620); and colour tone (A420/A520).

In the first 10 days of October, at commercial harvest time, all bunches of each plant in the selected subplots (40 plants per treatment) were collected to determine bunch weight and number per plant.

2.3. Data Analysis

2.3.1. Statistical Analysis

All data are presented as means \pm standard error and were statistically analysed by ANOVA according to a completely randomised design with four and two replications for grape and olive orchards, respectively. We adopted Tukey's HSD test to compare the means [40].

2.3.2. Satellite Vegetation Indices

The research compared the conventional vegetation indices typically derived from Landsat 8-Operational Land Imager and Sentinel-2-MultiSpectral Instrument data. The indices used include: (i) the Chlorophyll Index Red-Edge (CIRE) described by Gitelson and Merzlyak [41] as a good indicator of assessing production potential and understanding the nutrient status, stress due to water, and disease outbreak, etc.; (ii) the soil-adjusted vegetation index (SAVI) to assess vegetative cover where crop cover is low (e.g., grapes) or in arid regions [42]; (iii) the normalised difference vegetation index (NDVI) to estimate the density of green on an area of land [43]; (iv) the enhanced vegetation index (EVI2) to quantify vegetation greenness (compared to NDVI, EVI is more sensitive in areas with dense vegetation and it corrects for canopy background noise and atmospheric conditions) [44]; (v) the normalised difference red edge index (NDRE1) to assess N status and canopy density as indicators of crop health [41]; and (vi) the normalised difference red edge index (NDRE2) which is more accurate than the previous [45].

3. Results

The foliar biostimulant application differently affected the responses of the two crops studied.

3.1. First Experiment

Table 3 reports olive yield and olive oil chemical characteristics. The olive yield in the treated plots, although not significant, tended to be higher than in the control, with an average increase of 7.6%. Moreover, some oil chemical characteristics showed statistically significant differences. In particular, the oil acidity was significantly (p = 0.05) higher in the untreated control (0.23%) than in the treated plots (0.21%). Meanwhile, biostimulant application significantly (p = 0.01) increased total polyphenol content compared to the control. The increment in total polyphenols content was 3.6 times higher in treatment with the biostimulant than in the untreated control (Table 3).

Table 3. Yield production at harvesting time and chemical parameters of the oil §.

Treatments	Yield kg plant ⁻¹	Acidity %	No. Peroxides meq O ₂ kg ⁻¹	K232	K270	Total Polyphenols mg kg ⁻¹
Untreated control	75.27 a A	0.23 a A	5.95 a A	1.72 a A	0.09 b A	50.50 b B
Biostimulant	80.85 a A	0.21 b A	6.20 a A	1.78 a A	0.13 a A	181.00 a A

[§] The values in each column followed by a different letter are significantly different according to Tukey's HSD test; with lowercase letters, significance is at $p \le 0.05$ level and with uppercase letters, significance is at $p \le 0.01$ level.

In Figure 3, the monitored satellite indices between 24 May 2021, before the biostimulant application, and 31 October 2021, at the end of the agricultural season, are reported. Olive plants' vegetation, chlorophyll, and N status (CIRE, SAVI, EVI2, NDVI, NDRE1, and NDRE2) showed a net increase in the treated plots compared to the untreated plots. The



difference in indices' time-series means and standard deviations confirmed this increase (Figures 4 and 5), yet it was not statistically significant.

Figure 3. Comparison of (2A) CIRE, (2B) SAVI, (2C) EVI2, (2D) NDVI, (2E) NDRE1, and (2F) NDRE2 on treated and untreated olive plots in 2021.



Figure 4. Satellite indices time-series mean comparison on treated and untreated olive plots.



Figure 5. Satellite indices time-series standard deviation comparison on treated and untreated olive plots.

3.2. Second Experiment

After two biostimulant applications, the rachis length did not significantly differ from the untreated control (Table 4), whereas the third dose of the biostimulant, applied during the post-fruit set, induced a significant (p = 0.01) increase of 9.5%, on average, in bunch length compared to the control (Table 4).

Treatments	7 June 2021 Pre-Flowering Stage	21 June 2021 Post-Fruit Set	6 July 2021 Berries Beginning to Touch
Untreated control	10.8 a A	13.3 a A	13.7 b B
Biostimulant	11.3 a A	14.5 a A	15.0 a A

Table 4. Effect of biostimulant on rachis length (cm) ξ .

^{ξ} The values in each column followed by a different letter are significantly different according to Tukey's HSD test, with lowercase letters significance is at $p \le 0.05$ level and uppercase letters significance is at $p \le 0.01$ level.

The number of bunches was not significantly different between the treatments at harvest, whereas the bunch weight in the treatment with the biostimulant significantly increased (p = 0.05) compared to the control (Table 5).

Table 5. Effect of biostimulant on fruit yield at harvesting time *.

Treatments	Number of Bunches Plant ⁻¹	Bunch Weight (Std) (g)
Untreated control	34.85 a A	195.1 (89.6) b A
Biostimulant	34.3 a A	214.4 (97.3) a A

* The values in each column followed by a different letter are significantly different according to Tukey's HSD test, with lowercase letters significant at $p \le 0.05$ level and uppercase letters significant at $p \le 0.01$ level.

All variables related to the quality of grape pulp, determined in the four investigations carried out from fruit set to berry ripening, did not show any statistically significant difference (Figures 6–9). In particular, at harvest, the content of reducing sugars, glucose, fructose, and total dry extract (Figure 6) was, on average, 217, 108, 109. and 239 g L⁻¹, respectively. In any case, fifteen days before harvesting (at 20 September 2021), the values of these variables were similar to those recorded at harvest in the treatment with the biostimulant. In contrast, in the control, they tended to increase. This behaviour was also confirmed by the °Brix values (Figure 4) that increased in the last fifteen days before harvesting by 3% in the untreated control. In contrast, it remained almost unchanged (+0.6%) in the biostimulant treatment. In addition, pH value, total acidity, malic, tartaric gluconic and citric acid, and potassium content were similar in both treatments and during the ripening of the berries (Figures 7–9).



Figure 6. Evolution of reducing sugars, glucose, fructose, and total dry extract in the grape pulp during berry ripening (2021 season).



Figure 7. Evolution of total acidity, total sugar content (°Brix), and pH in the grape pulp during berry ripening (2021 season).



Malic acid — Tartaric acid — Gluconic acid — Citric acid — Potassium

Figure 8. Evolution of acids and potassium content in the grape pulp during berry ripening (2021 season).



Figure 9. Absorption spectra and colour tone variation during the 2021 season.

The monitored satellite indices in the grape orchard between 24 May 2021, before the biostimulant application, and 31 October 2021, at the end of the agricultural season, are reported in Figure 10. Although not significant, the vegetation, chlorophyll, and N status (CIRE, SAVI, EVI2, NDVI, NDRE1, and NDRE2) showed a slightly higher rate in the treated plots than in the untreated. After the third biostimulant application, the differences between the two treatments were more evident in September. The indices' time-series means and standard deviations confirmed this trend (Figures 11 and 12).



Figure 10. Comparison of (7A) CIRE, (7B) SAVI, (7C) EVI2, (7D) NDVI, (7E) NDRE1, and (7F) NDRE2 on treated and untreated grape plots in 2021.



Figure 11. Satellite indices time-series mean comparison on treated and untreated grape plots.



Figure 12. Satellite indices time-series standard deviation comparison on treated and untreated grape plots.

4. Discussion

Grapes (*Vitis vinifera* L. cv. Montepulciano) and olives (*Olea europaea* L. cv. Coratina) are important perennial crops that are well-adapted to the environmental conditions prevailing in the Mediterranean Basin. Since the consumption of these products is increasing, both as table fruits and industrial fruits (the viticultural and olive oil industries), sustainable practices are essential for modern agriculture to reduce environmental externalities while increasing crop yield. Therefore, biostimulant use can provide sustainable practices for farmers since their application could (i) improve nutrient use efficiency and, consequently, (ii) minimise fertiliser losses, (iii) increment total yield, and (iv) obtain a high-quality product.

This study shows that biostimulant application positively influences the agronomic performances of olive and grape plants in terms of olive oil quality improvement and grape yield quantity increase. This was also confirmed by the monitored satellite indices and is in line with the scientific literature [13].

Specifically, the lowest acidity and highest total polyphenol content observed in the treatment with biostimulant improved the olive oil shelf life. Moreover, the total polyphenol content is an essential parameter in olive oil quality due to the antioxidant effects of polyphenols. Many factors may affect fruit production and physiology and, consequently, olive oil quality, like climatic conditions, cultivar, agronomic practices, fruit ripening, and

harvest conditions [46–48]. Several studies reported the positive effects of biostimulant application on olive yield and oil quality [31,49–51]. In particular, Zouari et al. [50] observed that the foliar application of biostimulants could increase the oil polyphenol content over two consecutive growing seasons.

In grapevines, the three biostimulant applications mainly affected the yield and not pulp quality. Previous studies stated that the effects of biostimulants depend on several conditions, including the application rate, timing, and number of applications [51,52]. During the post-fruit set, the third biostimulant application seemed to positively affect bunch development and, consequently, the marketable yield. Indeed, biostimulant use in table grapes could change bunch morphology when applied during the development and growth of inflorescences and fruits [53–55]. Frioni et al. [33] observed that, in some cultivars of red grapevine, the biostimulant did not influence the change of pH and acidity. At the same time, the total soluble solid was slightly and positively affected during the first part of the ripening process, which partially agreed with our results.

Moreover, our findings highlight that the seaweed foliar application could be considered a viable strategy to stimulate early fruit maturation. In fact, the content of reducing total sugars, glucose, fructose, and total dry extract in grape pulp reached the plateau fifteen days before harvesting. In contrast, these variables increased in untreated control until the harvesting. Other authors also observed a faster fruit yield in the early season, after four biostimulant applications from blooming to the early development of strawberry fruits [56]. Appropriate nutrient management is crucial for optimising crop production [57]. Biostimulants should be used to enhance nutrient uptake and stimulate stress-related tolerance mechanisms [58] because they contain substances and/or micro-organisms whose function is to stimulate natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality [59]. Seaweed extracts contain organic matter, mineral nutrients, and various hormones that contribute to plant growth, photosynthetic activity, and tolerance to biotic and abiotic stresses, thereby improving fruits' yield and quality [60,61]. They constitute a valuable and innovative tool to overcome nutrient limitations in different crop systems by enhancing plant resilience and improving nutrient uptake and assimilation [13,62,63].

Although it is a preliminary outcome, the results in both experimental fields confirm a general opinion that the foliar biostimulant applications could have beneficial effects on growth, yield, and quality. Still, crop responses could be unpredictable because they depend on crop characteristics, the specific phenological stage at the application time, the growing conditions, the number of applications, and timing. In fact, in our study, the biostimulant use caused a significant increase in grape yield but no effects on pulp quality. At the same time, in the olive orchard, the treatment improved oil quality without a significant yield increase. Consequently, high scientific and technical proficiency is necessary to determine and optimise the rates and timing of applications.

Therefore, our results are a first step towards understanding the numerous effects of seaweed extracts on plant responses. Consequently, further investigations should be carried out to gain a better understanding of the mode of action of the biostimulants and to assess the reliability of their application in the open field to allow accurate protocols to be established for their effective utilisation.

5. Conclusions

This paper reports the preliminary results of an investigation on using a vegetableand seaweed-based biostimulant extract applied to grapes and olives, two strategic crops grown in the Apulia region. The results indicate that the biostimulant affects the responses of the two crops differently. In fact, the biostimulant applications in grapes increased the yield quantity but not the quality. In contrast, the olive yield did not change between treatments but the oil quality improved with the biostimulant applications.

This finding is in agreement with previous studies that found beneficial effects of biostimulants applied to several herbaceous and perennial crops. The outcome also confirms the scientific literature, which discusses the difference in response according to changes in abiotic and biotic factors. Therefore, for adaptative modern agriculture to be resilient against climate change, in-depth studies are fundamental to clarifying the mechanisms of these natural substances and identifying the optimal application techniques. Such studies will support high scientific and technical professionals in determining and optimising the rates and timing of such applications.

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