

Research Paper

Vegetable Extracts for Adaptive Agriculture: A Preliminary Assessment in Italy

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Abstract:

To meet the United Nations sustainable development goals (UN-SDGs) and the European Green Deal, plant biostimulants have become a necessity in agriculture. In particular, seaweed-based biostimulants have received a greater acceptance for their several benefits in crop growth and yield. This study evaluates the effects foliar applications based on vegetables and brown algae extract (*Ascophyllum nodosum* (L.) Le Jol., on grapes and olive yield in two field experiments in the Apulia region known for its modern agricultural sector. In particular, grape-growing and olive production. At harvest, the yield performances were determined.

The results highlighted that the crop responses differed in grape and olive orchards. The biostimulant application determined significant increases in bunch development (+9.5%) and bunch weight (+10%) compared to untreated control. In the olive orchard, the yield was not significantly influenced by biostimulant application, whereas we observed quality improvement in olive oil in treated plants compared with the control. To better understand the mechanisms behind this difference, the research concludes by suggesting pursuing in-depth studies and high scientific and technical proficiency to determine and optimise the rates and timing of applications.

Keywords: vegetable extract – seaweed extract – agrosystems – resilience – Apulia (Italy)

1. Introduction

The two-way relationship between agriculture and climate change poses a serious constraint towards 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 in 2020 the “European Green Deal” to develop the member states’ economy sustainably without increasing resources deterioration (https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en). 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].

Thus, the available literature has emphasised the importance of agrosystems adaptation and resilience to convert climate risks into opportunities for transformative agriculture [4, 5] to mitigate climate change and its impacts on food security and availability. However, in climate sciences, adaptation and resilience are newly introduced terminologies widely debated within the scientific community [6]. In this context, we adopt the definition suggested by Nelson et al. [7], which has also

been considered in the second working group of the IPCC [8]. This cited literature distinguishes adaptation as a process of change in behaviours, tools and practices as an action and/or reaction to external stressors and stimuli to reduce vulnerability and resilience as a system's adaptive capacity. Unlike adaptation which is actor-centred, resilience, according to this literature, is more system-oriented and dynamic.

The available scientific evidence has documented the importance of organic and mineral nutrition in crop growth and health [9], soil fertility [10], and productivity [11]. However, reaching the peak of crop nutrient uptake, the new paradigm for sustainable agriculture, based on integrating scientific and technological advances in nutrition, puts soil, health and the environment at the centre to manage highly productive agrosystems [12, 13] efficiently. Furthermore, the main targets of the F2F strategy include the reduction of fertilisers by 20% and nutrient losses by at least 50% [3].

For this purpose, biostimulants represent a promising field in crop nutrition, fundamental to reaching sustainable agrosystems, resilient to climate change [12, 14, 15]. Biostimulants were first defined by Zhang and Schmidt [16]. More recently, Du Jardin [17] defined biostimulants 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 [17]. 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 [12, 18-20]. 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 to assess its effects.

Indeed, a handful of studies investigated the effects of seaweed and botanical extracts on plant metabolism and physiological health [21, 22] and their phytohormone-like activity [23]. More recently, studies involved gene expression analysis in highlighting the effects of seaweed and botanical extracts on crops' metabolic regulatory pathways [24]. Other scholars assessed the changes in soil properties (biological, physical and biochemical) and their relation with nutrients' uptake efficiency [25-27]. Furthermore, seaweed and botanical extracts enhance the performance of plants under abiotic stresses such as tolerance to freezing temperature stress and high temperatures [28, 29], drought, water and salinity stressors [30, 31]. Agronomic efficiency has also been subject to different assessment studies to evaluate seaweed and botanical extracts' effects on improving productivity, product quality and shelflife [15, 32, 33].

However, acknowledging that climate change effects vary in time, space and intensity [34], adaptation in agriculture is related to sustainable agriculture and should be case-specific and needs to be assessed and evaluated, considering specific field conditions [35]. Therefore, unlike previous studies, this research aims to evaluate the agronomic and organoleptic effects of seaweed and botanical extract produced at the CMI Roullier laboratories (Centre Mondial de l'Innovation Roullier). The experiment assesses different management practices of two strategic crops in the Apulia region's agriculture (Italy).

2. Material & Methods

The experiment is located in Brindisi province in the Italian Apulia region, known for its modern agricultural sector. In particular, Apulia is placed first among grape-growing areas of Italy and has 35.5% of the total olive area (**Table 1**). The climate is typically the Mediterranean, with mild winters with an average minimum temperature of 7.8°C and hot summers with an average maximum temperature of 30.6°C and 628 mm of average rainfall annually.

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) (Including grapes and table grapes)	614,958	92,038
Olive Agricultural Area (ha) (Including table and oil olives)	1,032,858	366,897

(Source: Istat [36])

2.1 Experimental Design

The field trials were performed in olive and grape orchards in Ostuni and Carovigno in Brindisi province (**Figure 1**). 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, and two untreated (**Figure 1**). The product was conceived in the CMI Roullier laboratories (Centre Mondial de l'Innovation Roullier) [21, 24] 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 1: Maps of the case study within the regional and local context.

2.1.1 First Experiment

The trial was carried out in 50 years old olive orchards in loamy soil at 30 m above sea level (**Table 2**). The orchard consisted of Coratina and Leccino cultivars, spaced 10.0 x 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.

Agricultural practices were similar for all plots in fertilisation, irrigation and phytosanitary applications. Treated plots in the olive orchard received two foliar applications, first at veraison and the second twenty days after. Applications were performed with a 1,000 L atomiser, equipped with six nozzles per side, each 2.5 mm 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 cv Montepulciano planted in 1991 in a tendon training system (**Table 2**). Plants spacing between rows and within the row was 2.5 m x 2.5 m with an East-West orientation and planting density of around 1,600 plants ha⁻¹. Two plots of 2000 m² were selected for each treatment within the experimental field.

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

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 (µS 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

2.2 Data Collection

2.2.1 First Experiment

In each plot of the olive orchard, we selected ten plants of cv Leccino for data collection, for a total of 20 plants/treatment. We determined yield at harvest (first decade of November) and oil extracted from representative fruits of each plot (10 kg). Free fatty acids (acidity), number of peroxides and spectrophotometric examination in the ultraviolet (K232, K270 and DK) were carried out in accordance with Reg. CEE 2568/1991 11/07/1991 GU CEE L248 05/09/1991 and subsequent modifications. Finally, the total polyphenol content in the olive oil was determined by a colourimetric reaction with the Folin Ciocalteu reagent.

2.2.1 Second Experiment

In each plot of the grape orchard, we identified two sub-plots, each of 10 plants for a total of 40 plants/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 pre-flowering, and post-fruit-setting. In addition, from veraison to harvesting time, four samplings each of 1 kg were randomly performed, taking portions of bunches (3-4 grapes) from each sub-plots on each plant at different exposure and position.

The grape juice obtained from each sample (4 samples/treatments) 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 color tone (A420 / A520).

In the first decade of October, at commercial harvest time, all bunches of each plant in the selected sub-plots (40 plants/treatments) were collected to determine bunches' weight and number per plant.

2.3 Data Analysis

2.3.1 Statistical Analysis

All data are presented as means ± standard error and/or were statistically analysed by ANOVA according to a completely randomised design with four and two replication, respectively, for grape and olive orchard. We adopted the Tuckey LSD Test to compare the means.

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 included: *i*) the Chlorophyll Index – Red-Edge (CI_{RE}) described by Gitelson and Merzlyak [37] as a good indicator of assessing production potential, 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 [38]; *iii*) the Normalised Difference Vegetation Index (NDVI) to estimate the density of green on an area of land [39]; *iv*) the Enhanced Vegetation Index (EVI2) useful in quantifying vegetation greenness; compared to NDVI, EVI is more sensitive in areas with dense vegetation, and it corrects for canopy background noise and atmospheric conditions [40]; *v*) the Normalised Difference Red Edge Index (NDRE1) to assess N status and canopy density as indicators of crop health [37], and *vi*) the Normalised Difference Red Edge Index (NDRE2) which is more accurate than the previous one [41].

3. Results

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

3.1 First Experiment

The olive yield in the treated plots, although not significant, tended to be higher than control (+7.6%) (Table 3). Moreover, we observed significant differences for some oil chemical characteristics. In particular, the oil acidity was significantly higher in untreated control than in the treated plots (p=0.05). Biostimulant application significantly increased total polyphenol content in respect of control (p=0.05) (Table 3). Total polyphenols content is an essential parameter in olive oil quality due to their high antioxidant effects; in our research, the oil from the drupes treated with biostimulant had a polyphenol content 3.6 times higher than the untreated one.

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

Treatments	Yield kg plant ⁻¹	Acidity %	N° 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

Yield potential depends on the photosynthesis efficiency of the crop, and the literature associates phenolic compounds' abundance with the photosynthetic performance of vegetation [42, 43]. For this purpose, we monitored satellite indices between 24/05/2021, before the biostimulant application, until 31/10/2021, the end of the agricultural season. The 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 (**Figure 2**). This increment explains the yield difference and the concentration of phenolic compounds observed in the treated plots.

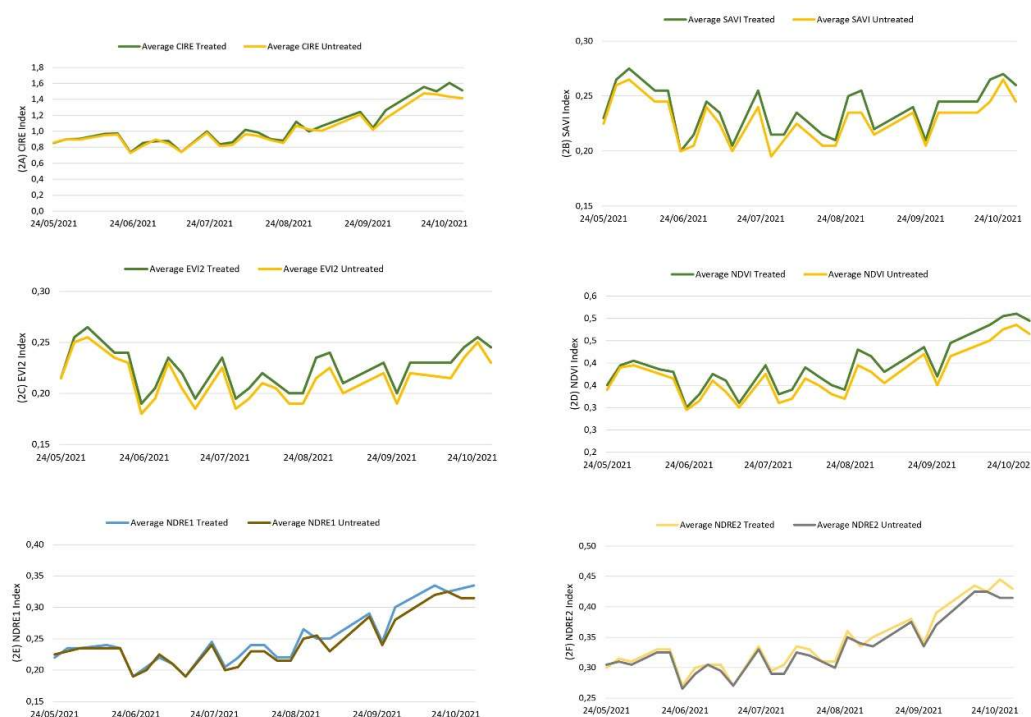


Figure 2: Comparison of (2A) CIRE, (2B) SAVI, (2C) EVI2, (2D) NDVI, (2E) NDRE1, and (2F) NDRE2, on treated and untreated olive plots.

The olive results highlighted that biostimulant use could mainly improve oil quality. Moreover, the lowest acidity and the highest polyphenol content improve the oil's shelf life. Many factors may affect fruit production and physiology and, consequently, olive oil quality, like climatic conditions, cultivar, agronomic practices, fruit ripening and harvest conditions [44-46]. Appropriate nutrient management is crucial for optimising crop production [47]. Biostimulants should be used to enhance nutrient uptake and stimulate stress-related tolerance mechanisms [48] because they contain substances and/or micro-organisms whose function is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality [49].

Several studies reported the positive effects of biostimulant application on olive yield and oil quality [33, 50-52]. In particular, Zouari et al. [51] observed that the foliar application of biostimulant could increase the oil polyphenol content during two consecutive growing seasons.

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 stage, induced a significant increase of the bunch length ($p=0.01$) of 9.5%, on average, compared with the control (**Table 4**).

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

Treatments	07/06/2021 pre-flowering	21/06/2021 Post-fruit set	06/07/2021 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

Previous studies stated that the effects of biostimulants depend on several conditions, including the application rate, timing and number of applications [53, 54]. During the post-fruit set, the third biostimulant application seems to positively affect bunch development and, consequently, the marketable yield. In fact, at harvest, the number of bunches was not significantly different between the treatments. Still, the bunch weight in the treatment with the biostimulant increased significantly ($p=0.05$) compared with the control (**Table 5**). Several studies highlighted that biostimulant use in table grape causes changes in bunch morphology when applied during the development and growth of inflorescences and fruits [55-57].

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

No significant difference was found for all parameters related to the quality of grape pulp, reducing sugars, glucose, fructose, total dry extract, total acidity, total sugar content, pH (**Figures 3 and 4**), the evolution of acids, potassium content (**Figure 5**), absorption spectra and colour intensity (**Figure 6**), obtained in the four investigations carried out from fruit set to berries ripening. The finding is partially in agreement with that obtained by Frioni et al. [58], that observed that, in some cultivars of red grapevine, the biostimulant did not influence the evolution of pH and acidity, while the total soluble solid evolution was slightly positively affected during the first part of the ripening process.

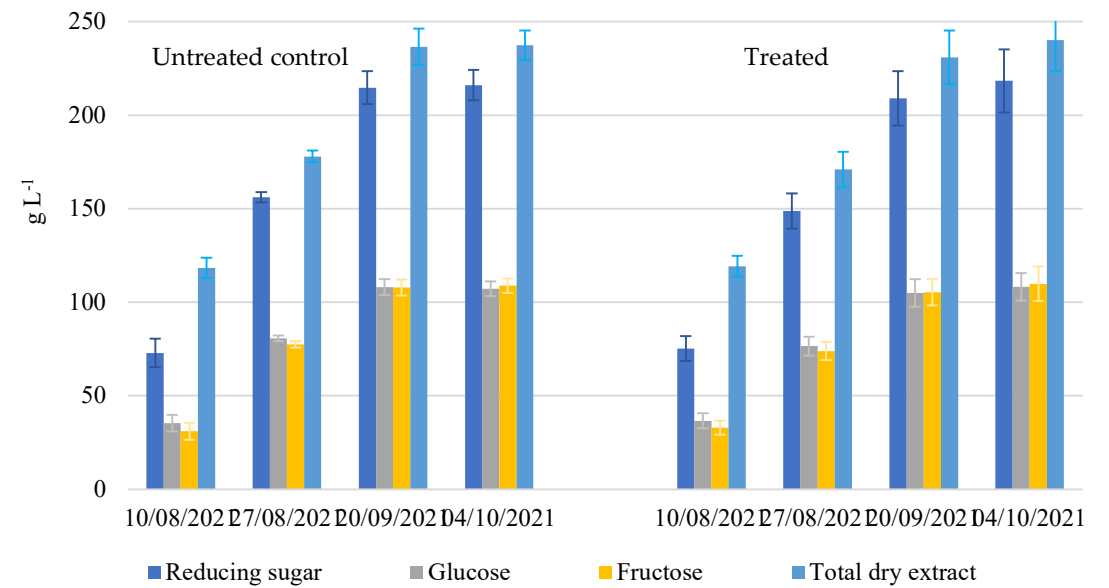


Figure 3: Evolution of reducing sugars, glucose, fructose, total dry extract in the grape pulp during the berries ripening.

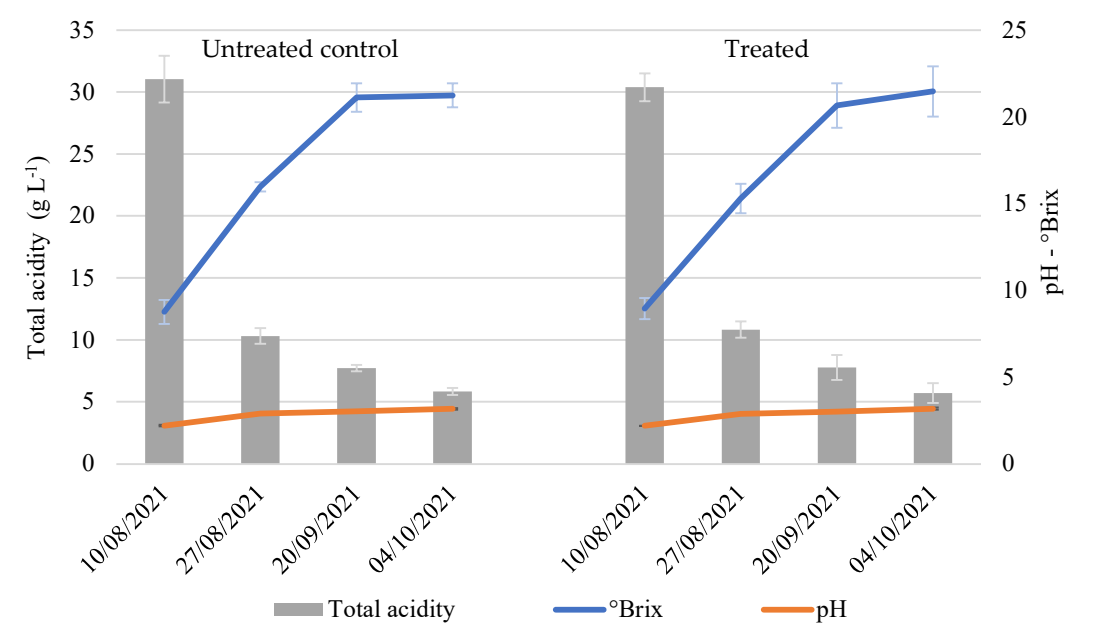


Figure 4: Evolution of total acidity, total sugar content (°Brix), pH in the grape pulp during the berries ripening.

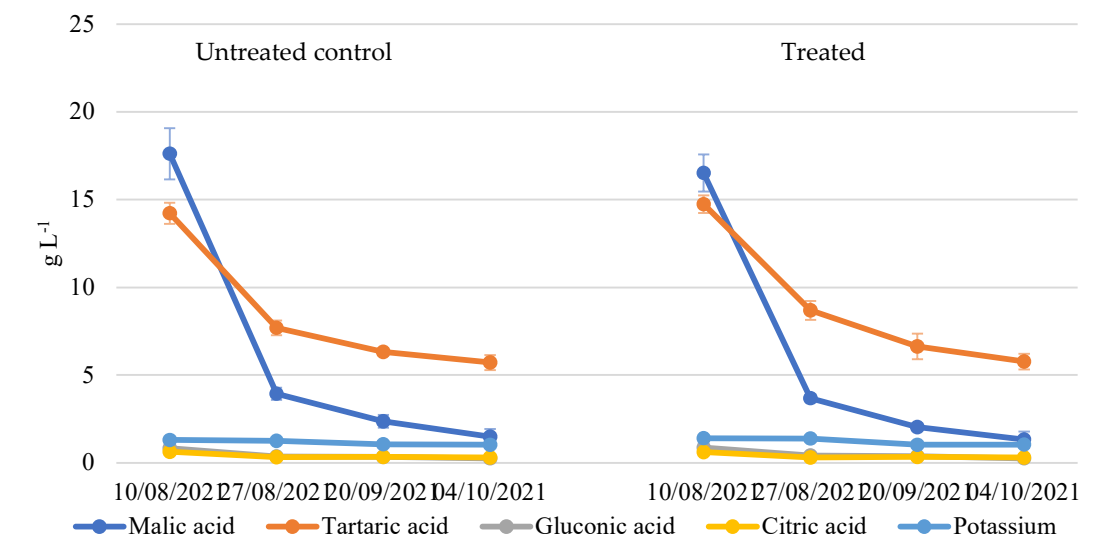


Figure 5: Evolution of acids and potassium content in the grape pulp during the berries ripening.

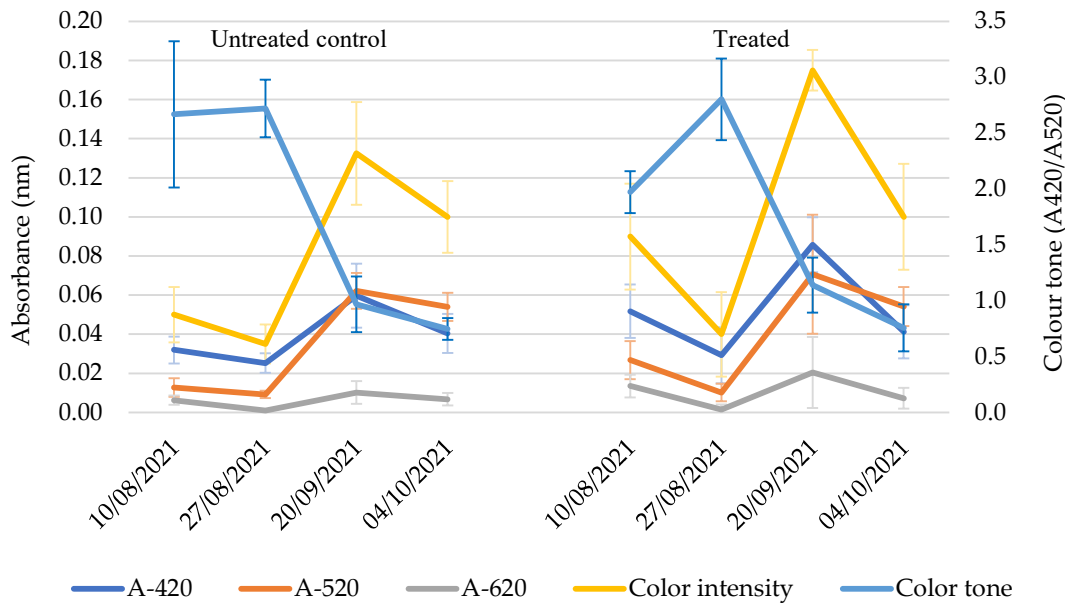


Figure 6: Absorption spectra and colour tone variation during the season.

We also monitored satellite indices in the grape orchard between 24/05/2021, before the biostimulant application, until 31/10/2021, the end of the agricultural season. The vegetation, chlorophyll and N status (CIRE, SAVI, EVI2, NDVI, NDRE1 and NDRE2) also showed, in this case, higher rates in the treated plots compared to the untreated plots (**Figure 7**), which emphasises the statistical results from the field experiment.



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

4. Discussions

Grapes (*Vitis vinifera* L. ssp. sativa) and olives (*Olea europaea* L.) are important perennial crops, well adapted to the environmental conditions prevailing in the Mediterranean Basin. Since the consumption of these products both as table fruits and industrial fruits (viticultural and olive oil industry) is increasing, 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.

Seaweed extracts contain organic matter, mineral nutrients and various hormones that can contribute to plant growth, photosynthetic activity and tolerance to biotic and abiotic stresses, thereby improving the fruit yield and quality [59, 60]. They constitute a valuable and innovative tool to overcome nutrient limitation in different crop systems by enhancing plant resilience and improving nutrient uptake and assimilation [15, 61, 62].

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 implicated a statistically significant increase of grape yield but no effects on quality, while in the olive orchard, it improved oil quality without a statistically significant yield increase, which requires high scientific and technical proficiency to determine and optimise the rates and timing of applications.

Moreover, 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. [63] observed positive effects of biostimulant application on yield performance in an apple orchard in two consecutive years, highlighting that the results obtained were independent of the impact of the seasonal climatic conditions. Similarly, Frioni et al. [58] 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). Whereas in other studies, the authors observed different behaviours of the crops treated with biostimulant due to different climatic conditions [51, 64]. In particular, Gutiérrez-Gamboa et al. [64] observed that in grapevine, the seaweed applications 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. [65] stated that not all microalgae species have significant biostimulant activities on plant growth, suggesting that the biostimulant properties are due to “specie-specific” metabolites produced by particular microalgae species.

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. Conclusion

This study reports the results of a year of investigation on using a vegetable and seaweed-based biostimulant extract applied to grapes and olives, two strategic crops grown in the Apulia region. The results indicate that the biostimulant affected 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.

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

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