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Review

Sustainable Olive Culture under Climate Change: The Potential of Biostimulants

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Abstract: Climatic extreme events, like droughts, heatwaves, and floods are becoming recurrent and represent a threat to agriculture, lowering plant growth and productivity. The Mediterranean region is a climate-change hotspot, where traditional agricultural systems, like olive groves, are particularly challenged. Both the traditional and intensive systems of olive culture coexist in the Mediterranean. Both systems differ in their demands for water and agrochemicals, but nowadays, the global inputs of agrochemicals and irrigation have increased to achieve high productivity and profitability. Finding sustainable alternatives to maintain high productivity under the ongoing climate change is urgent to meet the EU-Farm to Fork strategy and climate neutrality. Candidate eco-friendly alternatives include biostimulants. These are substances or microorganisms, that activate signaling cascades and metabolic processes, increasing plant yield, quality, and tolerance to stressors. These benefits include a better growth, nutritional status and water availability, leading to a decreased demand for irrigation and agrochemicals. In this review, we aim to present different types of biostimulants (e.g., seaweed, protein hydrolysates, humic substances, microorganisms and nanomaterials), their mode of action and benefits in agriculture. We also explore the current state-of-the-art regarding the use of biostimulants in olive culture, and their potential benefits to increase tolerance to (a)biotic challenges.

Keywords: *Olea europaea*; biostimulant; seaweed; protein hydrolysates; humic substances; microorganisms; nanoparticles

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1. Introduction

Agriculture is facing unprecedented challenges due to the high food demand for the increasing world population, high competition over the scarce natural resources, and climate change threats [1]. The increasing global population exerts high pressure on the arable land to increase productivity but at the expense of high uses of agrochemicals (e.g., fertilizers and pesticides) [1]. In addition, climate change, particularly drought and heat waves are threats to food security and production all over the world [2,3] and are predicted to become more frequent and intense leading to vast socioeconomic and biodiversity losses [2,3]. Under this scenario, several changes must be implemented, starting with developing more sustainable agricultural practices without increasing resource deterioration. Within the Farm to Fork strategy of the European Union (EU), several targets to adapt to climate change and to increase crops' resilience were established [4]. The Farm to Fork strategy aims to reduce the use of fertilizers by 20% and pesticides by 50%, by implementing sustainable agricultural practices [4]. To achieve these goals, new strategies evolved in the last decades to improve agriculture's sustainable production. Biostimulants are a promising strategy to reach sustainable agricultural practices, since are based on the use of (preferably) natural compounds or microorganisms whose function in low levels promote plant growth-related processes, improve plant nutrient uptake and use efficiency, increase resistance or tolerance to abiotic stress, improve the quality of crop-derived products, and/or improve soil quality and health [5,6].

Biostimulants differ from the synthetic substances widely used in agriculture to enhance crop productivity, like inorganic nitrogen and phosphorous fertilizers, pesticides and others agrochemical [7]. Biostimulants act in several molecular and physiological processes of the plant boosting its performance. Fertilizers supply the plants with nutrients required for cells to sustain growth and metabolism, and pesticides help in the control (extermination) of pests and weeds, but their imbalanced use impact negatively various components of water, air and soil ecosystem as well as human health [7]. In the case of biostimulants, since most of the components are from natural origin, their impact in the environment is reduced [5,6]. Biostimulants are reported to be biodegradable, non-polluting and non-hazardous/non-toxic to several organisms, particularly at the recommended application rates [8]. This aspect is important from an environmental point of view. Therefore, the use of biostimulants in agriculture is in line with the demand for more sustainability in the agri-food sector, and these substances or microorganisms are suitable for organic and/or conventional farming [8].

Several categories of biostimulants have been described, but some of them still need a more comprehensive assessment in agriculture to answer all the requirements necessary for their wider use. The most used biostimulants are the seaweeds, protein hydrolysates, humic substances and microorganisms, and recently a new type of biostimulant have been described, the nanoparticles or nanomaterials [5,6]. Various studies are available on the advantages (e.g., growth promotion and stress tolerance) of using these biostimulants on crop species, such as rice, soybean, maize, tomato, grapevine, orange, and lemon trees [9]. However, their potential benefits in olive culture, one of the most important economic sectors in the Mediterranean basin, is less known.

2. Olive Tree and Culture Systems

The olive tree (*Olea europaea* L.) belongs to the genus *Olea* within the Oleaceae family, which comprises 600 species within 25 genera [10]. The *O. europaea* L. species is an evergreen tree presenting a medium size that can grow up to 10 m [11]. It is a xerophyte species, well adapted to the harsh environment conditions of the Mediterranean region. *Olea europaea*, subspecies *europaea*, includes the wild (*Olea europaea* subsp. *europaea* var. *sylvestris*) and the cultivated olive form (*Olea europaea* subsp. *europaea* var. *europaea*) [11].

This species is grown in the Mediterranean basin many years ago. The olive tree's existence goes back to the 12th millennium BC, and the cultivated olive has appeared in Asia Minor [11]. Archaeological findings from Ebla, Syria, mention oil production dating back to the 3rd millennium BC [11]. Over the years, the olive tree plantations increased mainly in Syria, Palestine, and Egypt, where oil was used in the lamps, and also for health purposes (used of oil to protect the skin against cracks and sunburns) [11]. The expansion of cultivated olive in the Mediterranean basin occurred mostly during the Roman Empire [11].

Nowadays, the olive culture comprises several socioeconomic benefits for the Mediterranean region. For instance, their products (table olives and olive oil) are increasingly recognized for their health benefits, which increased the demand by consumers, increase the producers' income, and the generation of employment [11–13]. Also, oliviculture and olive oil integrate the landscape and socio-cultural heritage of many Mediterranean countries, including in gastronomy [14]. The importance of this chain of value has thus raised the research and innovation dedicated to olive culture.

Europe dominates the Olive production and market. According to the EU agricultural outlook 2021-31, EU produces roughly 68% of the world's olive oil, with ~4 million ha mostly in the EU Mediterranean countries, with Spain and Italy, Greece and Portugal being top producers [11]. The EU also holds a major share of global consumption (47%), being Spain, Italy, Greece, and France in the top consuming countries (EC 2021) [11]. Table 1 shows the production of olive oil in the last years and their consumption in the Mediterranean countries. Beside olive oil, also table olives market has been increasing in the last years and the Mediterranean countries, particularly Spain and Egypt, are the main producers (Table 1) [11]. Olive fruits and oil are well known for their nutritional value. They are rich in lipids (particularly a high content of unsaturated, monounsaturated and polyunsaturated fatty acids), on other minor compounds like phenols, sterols, squalene, tocopherol, proteins and pigments [15].

In the Mediterranean region, there are two major models of olive cultivation, the traditional and the intensive and/or high-density olive groves model, although with different cultivated area (much more for the traditional olive culture). In the traditional model, where olive trees are dryland farming or irrigated, with 40 to 240 trees/ha, the use of phytopharmaceuticals and fertilizers is reduced [16]. In some way, the grove is managed less industrially, farmers often only cut the weeds to facilitate access to the olive trees. Also, biodiversity is higher in this model of production [17,18]. In the intensive and/or high-density olive groves model the density of the trees per hectare varies between 200 to 2500/ha [12]. In these orchards, trees can be planted in hedgerows, the trees are irrigated [e.g., olive water requirement in intensive culture: around 800 mm ha $^{-1}$ year^{-1} (source: International Olive Oil Council)], and phytopharmaceuticals and fertilizers are applied under control measures. This model of culture is oriented to be super productive and with high income [16]. The biodiversity in this model of production is usually poorer than compared in the traditional model [17,18].

Besides requiring different cultivation practices, the intensive and/or high-density olive groves model and the traditional model of olive cultivation also require cultivars with different characteristics. Regarding the olive cultivars, more than 2600 cultivars are estimated to exist worldwide [19]. Of these, over 600 cultivars are certified and registered in the World Bank of Olive Germplasm established in Spain [20]. Few are the cultivars that proved to be suitable for intensive and/or super-intensive culture systems (e.g., 'Oliana', 'Sikitita', 'Lecciana', 'Arbequina', 'Arbosana' and 'Koroneiki') [21], while interest is boosting regarding an integrative characterization of the genetic resources provided by ancient cultivars (e.g., 'Cobrançosa', 'Negrinha', 'Manzanilla', 'Hojiblanca', 'Cornicabra', 'Castellana') to improve tolerance to (a)biotic stressors.

Table 1. Olive oil production (×1000 t) and consumption (% of world), and table olives production (×1000 t) in the Mediterranean countries in the last years (source: International Olive Oil Council) and main cultivars [19,22]. Na–data not available at the International Olive Oil Council.

Country	Olive Oil Production		Olive Oil Consumption	Table Olives Production		Cultivars
	2020/2021	2021/2022		2020/2021	2021/2022	
Spain	1389	1300	17	547	645	Arbequina, Arbosana, Aloreña, Alfafara, Blanqueta, Cornicabra, Empeltre, Farga, Hojiblanca, Lechín, Manzanilla, Morisca, Negral, Nevadillo, Picual, Picudo, Redondilla, Royal, Sevillenca, Verdeal de Vélez-Málaga and Villalonga
Greece	275	225	4	230	165	Anphissis, Chalkidiki, Conservolia, Kalamon, Koroneiki, Kolybada, Liano-lia, Mastoidis, Megaritiki, Ntopia Atsicholou, Ntopia Pierias, Petrolia Serron, Smertolia and Chrysophylli

Table 1. *Cont.*

Country	Olive Oil Production 2020/2021	2021/2022	Olive Oil Consumption	Table Olives Production 2020/2021	2021/2022	Cultivars
Italy	274	315	15	80	59	Ascolana, Bella di Cerignola, Biancolilla, Bosana, Canino, Carolea, Casaliva, Casasene, Cellina di Nardò, Cima di Melfi, Coratina, Frantoio, Giarraffa, Leccino, Maurino, Mele, Moraiolo, Nocellara del Belice, Nocellara etnea, Ogliarola, Olivastra Seggianese, Pendolino, Peranzana, Razzola, Taggiasca and Termite di Bitetto.
Portugal	100	120	2	16	21	Azeiteira, Blanqueta, Cobrançosa, Cordovil, Carrasquenha, Galega, Lentisca, Madural, Verdeal and Redondil
Tunisia	140	240	1	Na	Na	Baroni, Chétoui, Chemlali, Oueslati, Chemlali Tataouine, Esraadki, Gerboui, Meski, Neb Jmel, Rkhami, Roumi, Rajou and Zalmati
Turkey	210	228	5	360	402	Ayvalik, Balıkesir, Domat, Erkence, Çakir, Halhali, Memecik, Memeli, Uslu, Izmir Sofralik, Gemlik, Kilis, Kiraz and Otur
Morocco	160	200	4	130	130	Picholine Marocaine, Picholine Languedoc, Dahbia, Haouzia, Menara and Meslala
Algeria	70	98	3	278	326	Aaroun, Albani, Aedli, Azeradj, 'Ballouti amzel, Blanquette, Bouchouk, Chemlal, Djbaili, Ferkani, Ferdel, Khadraya, Hamra, Limli, Mekki, Sigoise, Roulette and Zeboudj
Egypt	30	20	1	500	500	Wateken, Maraki, Wardan, Meloky, Sebhawy, Sinawy, Bez El Anza, Kosiem, Abou Monkar and Siwy

3. Impact of Climate Changes on Olive Trees Performance and Quality

Olive trees are known to tolerate a broad range of environmental stresses, including heat, drought, salinity and high levels of UVB [13,23,24]. For drought stress tolerance, olive trees developed several morphological adaptations and physiological and biochemical mechanisms, including some xerophytic morpho-anatomic characteristics [25,26]. Salt tolerance in olives is mostly related to efficient mechanisms of ion exclusion and retention of Na^+ and Cl^- in the root [27]. These stress adaptation strategies are already activated at the expense of plant energy, reducing plant development and growth.

The persistence and frequency of weather climate events due to climate change represent a threat to this culture, decreasing photosynthesis, yield, and changing fruit and oil quality [12,13,15]. Changes in temperature and precipitation patterns in spring and summer coincide with one of the most sensitive phases of the olive life cycle, the reproductive phases of fruit set and fruit development, leading to negative consequences for olive crop yields [28]. Drought and salinity may decrease the olive leaf water status and influence stomatal closure [29,30]. Water deficit may also compromise photosynthetic rates and photochemical efficiency [23,29,31]. Moreover, photosynthetic pigments are very sensitive to drought, decreasing their content [23]. Also, excessive heat decreases the net CO_2 assimilation rate, stomatal conductance, transpiration, and intercellular CO_2 concentration, and increases pigment contents in olives [32,33]. Moderate and high levels of UV-B rays increase the production of reactive oxygen species (ROS) in olives, leading to oxidative stress and consequent reductions in photosynthesis (light-dependent reactions and C assimilation), including decreases in RuBisCO activity, and in photosynthetic pigments [32,34–36]. Salt stress in olive reduce photosynthesis, chlorophyll levels, stomatal conductance and increase the production of ROS leading to lipid peroxidation [24,27,30]. These alterations in olive performance also affect productivity and quality [13]. Drought decreases fruit yield and oil accumulation and accelerates maturation [13]. Fruit metabolite content (phenolic and lipid compounds), as well as oil total phenols and sensory quality, can be increased by drought conditions [15]. However, mild effects of drought conditions on oil free acidity, peroxide value, and specific absorption coefficients (K_{232}, K_{270}, and ΔK) were reported [13,37]. High temperatures also change oil quality, reducing oil concentration in fruits, oleic acid, and total polyphenol contents [38,39]. Salinity may lead to increased phenolic compounds levels, such as tyrosol, hydroxyltyrosol, and acids vanillic, coumaric, and ferulic [30]. Some reports pinpoint that salinity induced some effects on oil composition, but no major adverse effect on fatty acid composition were found [30].

4. Sustainable Strategies to Improve Olive Culture under Climate Change Conditions: Biostimulants

The intensive and/or high-density systems of olive culture have been increasing in the last years, as this model offers a better cost/benefit for the producers. However, the cost for the environment is often neglected. As this model of olive production uses more fertilizers and pesticides, increase soil erosion and the rates of soil fertility loss, degradation of habitats and landscapes, and over-exploitation of vulnerable and scarce water resources [40]. Thus, it is primordial to establish a commitment in terms of the socioeconomic benefits without neglecting the ecosystem crucial for the generations to come.

Sustainable olive culture practices must address the challenges posed by climate change, and the intensive and high-density systems has to be replaced by less water-demanding practices like dry farming or the emerging partial root-zone drying [12,41] or even integrate the olive cultivation in agroforestry models. Also, other strategies to enhance olive culture under climate change conditions have been proposed, such as improvement of soil management, cover crop and pruning, use of alternative sources of irrigation water [42], and application of spray compounds to protect against extreme weather conditions (e.g., kaolin, salicylic acid and copper) [12]. Within the cover crop management practices, the use of seed-mix cover crops have been proposed, but it should be taken in to account that some types of cover crops can compete with the main crop for nutrients and/or water, particularly during the growing season [12]. However, in areas suffering from, for example, severe drought it may even turn out to be unsustainable the olive production as it demands irrigation [43–45]. Therefore, several changes in the olive culture practices must be implemented to produce safe and enough food without depletion of resources. Among the several emerging strategies to increase plant tolerance to biotic and abiotic stresses, biostimulants are a promising agronomic tool to achieve a sustainable agriculture [46]. Biostimulants are natural compounds or microorganisms that stimulate plant processes leading to improved nutrient use efficiency, growth and yield, and increased tolerance to biotic and abiotic stresses [47,48]. The positive effects of using biostimulants on plant growth and yield under biotic and abiotic stresses are described for several important horticultural crops (e.g., rice, soybean, maize, tomato, maize, grapevine, and spinach), fruit trees (e.g., orange and lemon trees) and ornamentals plants [9]. Biostimulants have also been proposed as candidates to enhance the phytoremediation of polluted environments [49]. Recent studies showed that plants treated with biostimulants (e.g., arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, silymarin-based biostimulant, and commercial biostimulants like Fertiactyl Pós ® and Megafol®) and growing in polluted sites with heavy metals or pesticides, showed higher capacity to growth and promoted phytoremediation [49].

In the last years, the market for biostimulants has been increasing at a rate of around 12% per year, and it is expected to achieve in 2026 a market value of around 6 billion dollars [50]. The success of these products is mostly related to the actual changes in agricultural and environmental policies that drive the use of sustainable alternatives to synthetic chemicals allied to the increase in climate change scenarios [50].

Mediterranean olive producers' countries have been implementing strategies to be more sustainable. Aligned with the Farm to Fork (F2F) Strategy, the EU aimed that the food systems become fair, healthy, and environmentally friendly. To be able to achieve these goals, agriculture must be neutral and have a positive impact on the environment, promote climate change mitigation and adapt to its impacts, promote biodiversity, ensure food security, nutrition, and public health, make sufficient, safe, nutritious and sustainable food access to everyone promoting fair trade and a fairer economic return [51]. EU countries when implementing those measures need to reduce, or even eliminate, the use of agrochemicals and pollutants, replacing them with natural alternatives. The biostimulants are alternatives able to maintain or even improve the nutritional value of soil (and consequently food) and promote and/or improve water management [5]. Moreover, the use of these compounds meets the F2F-EU goals, valorizing the native biodiversity, promoting strategies to reduce or eliminate alien species, and educating people to achieve those goals and improve food security [4].

The beneficial impacts of biostimulants led researchers to hypothesize that they may help the plant to better resist environmental stresses [5,52], namely heat and drought. The advantage of using these substances relay on their effectiveness in improving crop productivity and quality [52]. In line with the F2F strategy and to face the challenges of climate change, biostimulants thus emerge as promising alternatives to hazardous agrochemicals, putatively suitable for more sustainable agriculture. Additionally, these biostimulants are often considered to be of low cost [53], and may have multiple natural origins, including algae [52].

4.1. Types of Biostimulants and Mode of Action

Several types of biostimulants are available in the market, and they can be found in liquid, powder, or granular form applied in leaves (foliar spray) or/and around the root zone. Biostimulants can be classified according to the source of raw material into several groups, such as seaweed, protein hydrolysates, humic acids, nanoparticle and microorganisms (e.g., bacteria and fungi) [48]. More recently, nanomaterials were also integrated into the class of biostimulant as they can stimulate plant metabolic and physiological responses [54]. Despite the great interest and the several studies available on biostimulants, the natural complexity of these compounds makes it difficult to unveil their mode of action. Previous studies indicate that biostimulants act as priming agents, triggering molecular and physiological mechanisms that improve the plant's ability to defend faster and stronger against subsequent stresses [55].

4.1.1. Seaweeds

Seaweeds have been used as a fertilizer in agriculture for many years and provide a source of organic matter and nutrients [9]. Seaweed extracts represent around 1/3 of biostimulants' market worldwide [56]. Seaweeds include macroalgae comprising around 10,000 species, of which brown seaweeds with *Ascophyllum*, *Fucus*, *Laminaria* are the dominant groups [57]. Several seaweed species were already analyzed as plant biostimulants, belonging to the red algae (*Jania rubens*, *Gracilaria edulis*, and *Kappaphycus alvarezii*), green algae (*Ulva lactuca*) and brown algae (*Ascophyllum nodosum*, *Ecklonia maxima*, and *Laminaria*) [58–61]. The species, algae production and localization, and extraction methods of seaweed extracts influences their characteristics (color of the extract as well as the viscosity, solid content, particulate matter content and chemical composition) and hence its efficacy/bioactivity [62].

Seaweed extracts benefit plant growth and yield, root development, plant water status and water use efficiency, promote nutrient assimilation and increase resistance to abiotic (drought, salinity and heat), and biotic (pests and diseases) stressors [63,64]. These algae contain several compounds, such as polyphenols (e.g., phloroglucinol and eckol), hormones (e.g., auxins and cytokinins), polysaccharides (e.g., fucoidan, laminaran, carrageenan, and alginates) and vitamin K1 derivatives (e.g., kahydrin), that act in several central metabolic processes, like photosynthesis and antioxidant defence system. In particular, these biostimulants modulate the photosynthesis (adjusting the expression of genes related to RuBisCO activation, *RbCS1A* and *RCA*), control stomata aperture and induce protection against dehydration (through the overexpression of several genes, like *PIP1;2*, *bCA1, MYB60* and *RK2*, *RAB18 and LEA* group 2), and activate the expression of genes related to antioxidants (e.g., ascorbate) and antioxidant enzymes (e.g., SOD, CAT and APX) that help to control the levels of reactive oxygen species (ROS), reducing lipid peroxidation [9,45,57,59,65]. These biostimulants also stimulate the phenylpropanoid pathway as well as flavonoids biosynthesis, contributing to oxidative stress alleviation [9,45].

4.1.2. Protein Hydrolysates

Another type of biostimulant very used in agriculture includes animal- or plant-based hydrolysed proteins. Protein hydrolysates are usually derived from by-products of agro-industrial activities and are a combination of amino acids, polypeptides, and oligopeptides obtained by chemical, thermal, microbial, or enzymatic hydrolysis [66]. Protein hydrolysate biostimulants derived from collagen are rich in proline and glycine, whereas glutamic acid is more abundant in those derived from plant sources [67]. Protein hydrolysates derived from animal sources are richer in nitrogen (9–16% total dry matter) and have a lower release rate [61].

The action mechanism of PH involves stimulation of the plant antioxidant defense system and the photosynthesis. Concerning the antioxidant protection, PH upregulate the expression of phenylalanine ammonia lyase (PAL), heat shock proteins (e.g., *HSP16.9, HSP22* and *HSP116.9*) and dehydrins (e.g., *DHN2*, *DHN2*, *DHN4*, *DHN13* and *DHNCCor410*) genes, increase the antioxidant enzyme activities (SOD and APX), and the production of stress defence metabolites like terpenes, carbohydrates, amino acids and flavonoids, that control ROS overaccumulation, protect proteins and increase membrane stabilization [68]. Moreover, PH promote the photosynthesis and growth by stimulating pigment synthesis and protein production [69].

Beside plant performance, PH are also described to promote fruit productivity, growth, and quality in several crops, like tomato, papaya, passionfruit, corn, kiwifruit and pepper [70,71]. PH improve fruits nutritional level, increasing glucose, ascorbate, carotenoids, and stimulate secondary metabolism enzymes and consequently secondary metabolites production, like thymidine and flavonoids levels [70,71]. These beneficial effects of PH are particularly notorious under limited water availability (drought conditions), which is very relevant under the actual scenario of climate change.

4.1.3. Humic Substances

Biostimulants based on humic substances (HS) contain fulvic and/or humic acids, which are the major components of soil organic matter [58,72]. Humic substances biostimulants result from the decomposition processes of animals and plants, or the activity of soil microbes [48,73]. Humic substances are mostly composed of small amphitatic molecules that can form (supra)molecular aggregates in solution. In plants, the application of HS (e.g., extracted from leonardites or derived from vermicompost) activate several antioxidant defences like, antioxidant enzymes (e.g., increase the activity of SOD, CAT, APX and Gr), antioxidants (e.g., tocopherols, phenolic compounds, ascorbate and proline) and heat shock proteins (e.g., upregulate the expression of *HSP101*, *HSP81.8* and *HSP17.6A* genes) [74–76]. HS modulate the production of chlorophyll, regulate the expression of aquaporins leading to higher plant water status, and activate the nitrate metabolism which increase protein production and biomass accumulation [74,76]. Moreover, this king of biostimulants enhance root growth, nutrient uptake (e.g., can act as Fe-HS complexes), crop tolerance to environmental stresses [77], and even reduce the mutagenic activities of chemical compounds used in agriculture [78,79]. Current evidence suggests that the biostimulant effects of HS are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits). In addition, they can induce shifts in plant primary and secondary metabolism related to abiotic stress tolerance which collectively modulate plant growth and promote plant fitness [80]. When combined with other biostimulants, like microalgae, HS have synergic activity [66,67].

Concerning HS effects on fruit and vegetable productivity and quality, several studies demonstrated positive effects, even under stress conditions. In strawberry and muskmelon, HS application enhanced fruit yield, fresh weight, sugar content, firmness and diameter [80]. Moreover, HS increased aubergine, pineapple, potato and peach growth, tomato productivity, yield and sugar content, and enhanced grape berry and pepper size (width and weight) [80]. Vegetable crop growth and biomass production (e.g., in broccoli and chicory) are also stimulated by HS, mainly due to the enhancement of the nutrient use efficiency [80–82].

4.1.4. Microorganisms

Biostimulants based on microorganisms contain bacteria (e.g., plant growth-promoting bacteria-PGPB), yeast, and fungi (arbuscular mycorrhizal fungi-AMF) isolated from soils, plants, water, and/or organic materials [59,83] that interact with the plant roots, establishing mutual symbiotic associations or enhancing nutrient availability [48]. Within the PGPB biostimulants, those based on several rhizobacteria genera, like Azotobacter, Azospirillum, Bacillus, Pseudomonas, Rhodococcus, Rhizobium, Streptomyces, and Ochrobactrum, and within the AMF biostimulants, those derived from *Rhizophagus* spp., *Septoglycus viscosum*, *Claroideoglomus etunicatum*, and *Claroideoglo mus claroideum* are very used in agriculture [84]. Moreover, other fungal-based biostimulants derived from endophytic fungi, like the *Trichoderma* spp. (Ascomycota) and Sebacinales (Basidiomycota), are also well known and used in sustainable agriculture production [5].

PGPB action mechanism includes the activation of the antioxidant battery (e.g., antioxidant enzymes and metabolites), upregulation of stress protective genes (*sHSP*, *ERD15*, *CaPR-10*, *VA* and *Cadhn*), increase of the synthesis of 1-aminocyclopropane-1-carboxylate deaminase that promote growth, improvement of nutrient availability, osmoregulation, production of volatile organic compounds, and stomata control regulation [85–87]. Considering the AMF biostimulants action mode, they also involve the activation of the antioxidant enzymes (SOD and CAT) and metabolites (glutathione), and the downregulation of lipoxygenase [88]. Moreover, AMF also promote nutrient uptake, increase chlorophyll synthesis, osmoregulation and modulated the stomatal aperture by controlling ABA metabolism [88–90]. This kind of biostimulant regulate the expression of genes related to cellulose biosynthesis and cell growth (*XM_020312442.1* and *XM_020331230.1*) strengthening cell wall [91].

4.1.5. Nanoparticles or Nanomaterials

A new type of biostimulant includes nanoparticles/nanomaterials (with a size between 1 nm and 100 nm) [54]. These can be of metals (e.g., metal-oxides), metalloids (e.g., silicon), and non-metals, or of carbon (e.g., carbon nanotubes), organic (e.g., chitosan-based) or made of other materials (e.g., nanoclays). The nanoparticles (NPs) have a superior surface area to volume ratio compared to the non-nano-scale counterparts, improving their properties and significantly increasing their activity. NPs are often applied by foliar spray or in nutrient solutions, and can modify the plants growth and yield, and increase the nutraceutical quality of food crops, as well as their tolerance to stress [54]. Some NPs acting as biostimulants may be metallic such as TiO_2, ZnO_2, and Cu [54,92], of Si [93], and chitosan-based NPs [94]. For example, Regni et al. [95] showed that ZnO-NPs were beneficial to the olive tree in in vitro culture, while Cu-NPs showed activity against *F. oleagineum* and *Colletotrichum* spp., thus being beneficial to olive plants disease control [96]. Also, Si-NPs show potential for abiotic stress management, once alleviated drought effects on olive trees and modulated their response to the stress [93], besides of improving photosynthesis in drought stressed rice plants [97], and increasing the tolerance against salinity stress in cucumber by regulating proline and cytokinins [98].

The biostimulant properties of these compounds are associated with the type on NP, structure and nature of the materials [92]. For instance, due to the catalytic properties of TiO_2, they increased the content and expression of *LHCII b* genes in the thylakoid membrane, thus improving light absorption in chloroplasts, and accelerated RuBisCO activase, overall improving photosynthesis [92]. Moreover, this kind of NPs, can act as pro-oxidants and antioxidants modulating ROS signaling, can enhance nitrate reductase activity and nitrogen assimilation, leading to higher amino acids and protein production, and can promote growth [92]. In the case of ZnO_2NPs, they increase Zn availability in plants, enhancing chlorophyll and photosynthesis, and modulate the expression of genes and transcription factors (e.g., ARP, MPK4, MKK2, SKRD2, MYC, bHLH, EREB, HsfA1a, R2R3MYB, and WRKY1) associated with multiple responses, like physiological, hormonal, and abiotic stress tolerance [54,92].

Metal-NPs may slowly release, at controlled levels, nutrients (e.g., Fe, Mn, Zn, and Cu), thus providing a balanced transfer to the soils and food chain, in comparison with bulk fertilizer [99]. Besides the interest as plant stimulants, an increasing interest for nanomaterials in agriculture is related with their capability of controlled delivery of chemicals, pesticides or plant growth regulators [100].

4.2. Biostimulants Use in Olive Culture and Their Impact on Stress Tolerance

The use of biostimulants in olive culture is very limited, and few information is available about the advantages of the application of these compounds to alleviate the negative effects of biotic and abiotic stresses (Figure 1). Most of these studies were performed with microbial based biostimulants to control some pathogens responsible for disease that causes enormous productivity losses.

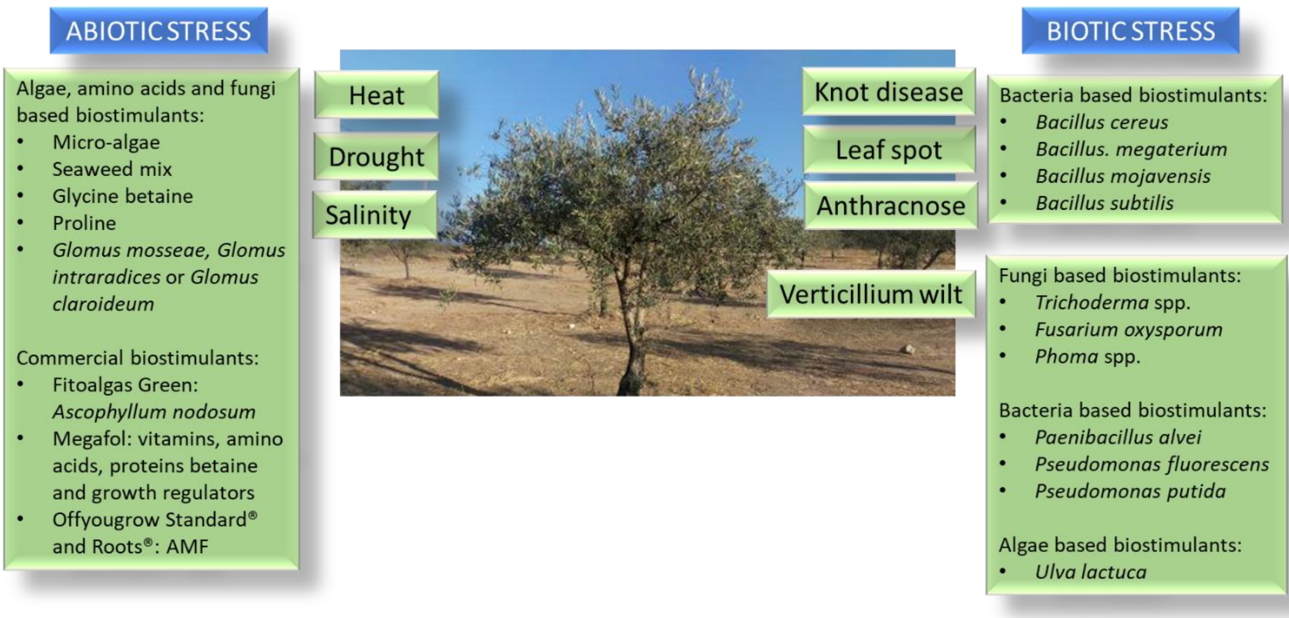

Figure 1. Biostimulants used to control abiotic and biotic negative effects on olive tree.

4.2.1. Biotic Stress Tolerance

The fungal disease, verticillium wilt of olive (VWO), caused by the fungal pathogen *Verticillium dahliae* Kleb is considered one of the most destructive diseases in olive groves causing enormous economic losses [101,102]. Verticillium wilt disease is very difficult to control, since the microsclerotia (the infective propagules of *V. dahliae*) can persist in the soil or in plant debris for several years and they can be spread by rain, irrigation and/or agricultural practices [102]. *V. dahliae* can penetrate the plant cell wall and infect the xylem vessels causing callose accumulation and obstruction of these vessels. This reduce the water transport and causes the wilt syndrome which symptoms are chlorosis, necrosis, defoliation, stunting, and in the worst case scenario plant death [102].

In the last decades, innovative approach based on natural products and microorganism (e.g., fungus, bacteria, seaweed and plant extracts) have been studied to control VWO [76,77]. Regarding the group of microbial biostimulants, several fungi and bacteria are described to be effective on the biocontrol of VWO (Table 2). The fungal-based biostimulants derived from the endophytic fungi *Trichoderma* spp. have been studied and the mechanisms of action are mostly based on the synthesis of antibiotics, production of hydrolytic enzymes that degrade the cell walls of the pathogen, competition for nutrients and space, and stimulation of plant defence system [103,104]. In the high susceptible olive cultivar 'Picual' (4 to 5-months-old plants), the treatment with *Trichoderma asperellum* (strains Bt3 and T25), or *Trichoderma harzianum* (strain GFP22) or *T. asperellum* combined with *Trichoderma gamsii* reduced the severity of VWO symptoms and *V. dahliae* growth [105–107]. Also, the fungus *Fusarium oxysporum* (strain FO12) showed a high effectiveness in the control of VWO disease in several olive cultivars, 'Picual', 'Arbequina', and 'Frantoio' (a susceptible, moderately susceptible, and moderately resistant to *V. dahliae*, respectively) by reducing the inoculum density of *V. dahliae* and VWO progress in the field [108]. Other fungus like *Phoma* spp. (PH01 and PH02) also showed potential to control VWO [107].

The bacteria *Paenibacillus alvei* (strain K165) is also effective in the control VWO on the susceptible cultivar 'Amfissis', by reducing the relative area under disease progress curve and the final disease severity index [109]. Other bacteria, like *Pseudomonas fluorescens* (PICF7) and *Pseudomonas putida* (PICP2 and PICP5) are also effective in the delay of VWO symptoms in the cultivars 'Picual' and 'Arbequina', decreasing the final disease incidence and severity, and in some case they also promote olive growth [110–112]. Moreover, *P. fluorescens* PICF7 induces systemic defence responses in stems and leaves of the cultivar 'Arbequina', upregulating several genes related to biotic and abiotic stress response (e.g., genes involved in plant hormones and phenylpropanoids biosynthesis such as PAL, ACL, LOX-2, and oxidative stress, like catalase) and inducing different transcription factors (e.g., bHLH, WRKYs and GRAS1) [113,114].

Concerning the use of biostimulants based on seaweeds and plant extracts in VWO control, few studies were conducted (Table 2). Salah et al. [101] reported that several polysaccharides extracted from the algae *Ulva lactuca*, such as ulvan, carrageenan, alginate and laminarin, stimulate natural defences in olive trees (cultivar 'Picholine Marocaine') against the VWO and limit *V. dahliae* spread. These polysaccharides seem to induce signalling processes through the modulation of secondary metabolic pathways [115]. The algal polysaccharides stimulate the activity of PAL and induce the accumulation of phenolics and lignin. Cell wall lignification increase plant resistant to the fungus mechanical penetration and membrane enzymatic degradation (caused by enzymes present in this fungus) [101]. Moreover, *U. lactuca* polysaccharides delayed the symptoms of senescence in olive, decreasing around 50% of vascular browning and reducing defoliation after elicitation. The use of biostimulants based on plant extract have been shown a low efficiency on the control of VWO. Varo et al. [107] reported that within the 44 plant extracts, only few induced some inhibitory effects on *V. dahliae* micellar growth. Future research on plant derived biostimulants is necessary, possibly exploring other plant extracts.

The biological control of other relevant olive diseases with microbial biostimulants have been less investigated (Table 2). For instance, several strands of Bacillus (*B. cereus* NB-4 and NB-5, *B. megaterium* NB-3, *B. mojavensis* A-BC-7, *B. subtilis* NB HNEB-1 and NB-6) showed positive effects on the control of the agents that cause the olive leaf spot (*Spilocea oleaginea)*, anthracnose (*Colletotrichum acutatum)* and knot disease (*Pseudomonas savastanoi pv. Savastanoi*) [116–120]. The *B. mojavensis* is able to control *P. savastanoi pv. Savastanoi* populations, through the decrease of production of necrotic tumours [116].

Isolates from *Bacillus* spp. have an inhibitory effect on *S. oleagina* conidial germination, controlling the olive leaf spot propagation [117].

Table 2. Biostimulants used in the control of some olive diseases.

Biostimulant	Pathogen	Effects	Reference
T. asperellum, T. harzianum, T. asperellum + T. gamsii	*V. dahliae*	• Reduce pathogen growth and help to decrease disease progression	[108]
F. oxysporum	*V. dahliae*	• Reduce the inoculum density of *V. dahliae* and disease progression	[107]
P. alvei	*V. dahliae*	• Reduce the disease progression curve and disease severity index	[109]
P. fluorescens and P. putida	*V. dahliae*	• Decrease disease incidence and severity, and promote olive growth • Upregulate genes related to (a)biotic stress response (e.g., genes involved in plant hormones and phenylpropanoids biosynthesis, like *PAL*, *ACL*, *LOX-2*, and oxidative stress, like catalase), and induce several transcription factors (e.g., bHLH, WRKYs and GRAS1)	[110–114]
Ulva lactuca	*V. dahliae*	• Limit pathogen spread • Stimulate the activity of PAL and accumulation of phenolic and lignin • Delay the senescence symptoms	[101]
Plant extracts	*V. dahliae*	• Some inhibitory effects on pathogen micellar growth	[107]
B. cereus *B. subtilis* *B. megaterium*	*S. oleaginea*	• Inhibit conidial germination	[117,119]
B. mojavensis	*P. savastanoi pv. Savastanoi*	• Decrease knot weights and pathogen population size • Production of less necrotic tumours	[116]
B. subtilis	*C. acutatum*	• Reduction of the incidence of latent infections on drupes	[120]

4.2.2. Abiotic Stress Tolerance

The use of several types of biostimulants (*Trichoderma* spp., micro-algae, seaweed mixtures and glycine) to improve olive performance to drought and high temperatures (heat) was described by Graziani et al. [121]. The biostimulants' positive effects were evident under water deficit conditions, with particular increase in the number of leaves and leaf area, total antioxidant capacity and phenolics contents (e.g., oleuropein and hydroxytirosol) in the *cv* 'Salella' treated with a mixture of microalgae and seaweed and with glycine betaine. Similar results were observed in the *cv* 'Galega' and 'Arbequina' treated with the commercial product Fitoalgas Green, based on *A. nodosum* [122]. This seaweed improved the olive physiological performance under drought conditions by maintaining the leaf water content and promoting the accumulation of starch and antioxidants (such as total phenols and flavonoids). Also under salinity stress, the treatment of olive plants (*cv* 'Arbequina') with the commercial biostimulant Megafol (based on vitamins, amino acids, proteins betaine and growth regulators) helped the plants to maintain a photosynthetic rate, leaf water availability and pigment content similar to the control (above those under salinity stress), and also stimulated the antioxidant system (e.g., SOD activity) leading to membrane protection (lower lipid peroxidation and H_2O_2 production) [123].

Greenhouse experiments with potted olive plants, *cv* 'Picual' (5-month-old), inoculated with *Pseudomonas* ssp. PICF6 and *Pseudomonas simiae* PICF7, together or individually improved the content of leaf flavonoids under drought and salinity stress [124]. Also, under salinity conditions (100 and 200 mM NaCl), the application of the amino acid proline to potted olive plants (2-years-old) enhanced stress tolerance by improving the leaf water status (leaf relative water content and water potential), upregulating the antioxidant enzymes (SOD, APC and CAT), enhancing the levels of chlorophylls, and improving the gas exchange parameters (photosynthesis, stomatal conductance and transpiration rate) [125]. On the other hand, olive roots colonization with *Glomus mosseae, Glomus intraradices* or *Glomus claroideum* enhanced nutrients uptake, leading to higher area root surface and biomass under saline conditions [126]. The use of two commercial mycorrhizal fungi (one containing the propagules of *Rhizophagus irregularis*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Funneliformis coronatum* and *Claroideoglomus claroideum*, and the other containing *Pisolithus tinctorius*, *Rhizopogon* spp., *Scleroderma* spp. and *Laccaria* spp.) to alleviate the drought summer stress conditions in young olive trees was very effective, improving growth and photosynthesis [127].

4.3. Other Strategies and Techniques to Improve Olive Stress Performance

Beside the use of biostimulants (Section 4.2), other strategies to improve olive tolerance to stress have been studied. For instance, melatonin, a hormone-like compound found in plants, has been used to enhance salt tolerance in several fruit crops including olive [128]. The application of melatonin in potted olive plants, *cv* 'Zard', exposed to salinity stress, improved olive leaf water status, biomass, and antioxidant system (CAT, SOD and APX). Moreover, these authors also described that this compound protected the photosynthetic pigments and the membranes (decreased the levels of H_2O_2 and lipid peroxidation), reduced Na^+/K^+ ratio, and increased the accumulation of proline, glycine betaine, soluble sugars, abscisic acid and indole acetic acid [128]. Also, the use of other compounds like the phytohormones salicylic acid and abscisic acid, and kaolin to alleviate olive summer stress has been investigated [129–133]. The foliar application of these compounds showed to be efficient to prevent the negative effects induced by summer stress in olive trees performance, by the modulation of several physiological and biochemical responses (e.g., increase of photosynthesis, soluble sugars and pigments).

A different approach using shoots of transgenic olive lines expressing *osmotin* gene was described by Silvestri et al. and Bashir et al. [134,135]. Osmotin is a cationic protein that seems to confer some tolerance to salinity stress, by enhancing the accumulation of osmolytes (e.g., proline and glycine betaine) [135]. Bashir et al. [135] used in vitro cultures of two olive *cvs* 'Canino' and 'Sirole' and two transgenic lines of 'Canino' (AT17-1 and AT17-2 expressing tobacco the *osmotin* gene) and treated them with NaCl solutions (ranging from 0 to 200 mM). The transgenic lines showed more tolerance to salinity (no injuries on growth and increase of the *O*-acetyl serine(thiol)lyase). Under drought stress condition, also the transgenic olive lines (*cv* 'Canino') expressing *osmotin* gene showed a higher growth and less lipid peroxidation [134].

Despite the results obtained in the above mentioned studies, the use of these strategies and technics (e.g., genetic manipulation) to improve olive performance and application in agriculture/orchards systems required more knowledge. Genetic improvement for (a)biotic stress tolerance is an interesting and promising strategy, but the long term direct and indirect implications associated are not well known. Moreover, the action mode of some of these substances differ from the biostimulants described above (Section 4.1). For instance, kaolin is applied in leaves forming a particle film that reduce light absorption and transpiration, nevertheless it raises some concerns related with photosynthesis impairments under specific environmental conditions (e.g., low radiation), as already highlighted by some authors [136].

4.4. Biostimulant Effect on Olive Fruits, Oil Yield and Quality

Similar to the reported for plant performance and growth, biostimulants can also affect olive fruit characteristics and oil composition [137]. However, some variability on the responses profile have been reported [137]. For instance, Chouliaras et al. [138] demonstrated that the application of seaweed extracts combined with boron improved the level of linoleic and oleic acids and peroxide value, but reduced the contents of palmitoleic, stearic and linoleic acids, and total phenols in olives from potted trees of the *cv* 'Koroneiki'.

The treatment of olive trees, *cv* 'Chemlali', with two biostimulants, Alcygol (seaweed with boron) and Nectar intense (based on proteins), changed the olive oil quality profile [139]. For the Alcygol biostimulant, an increase of carotenoids and a reduction of peroxide value and total phenols was observed, while with the use of Nectar intense, the monounsaturated fatty acids (MUFAs) and carotenoids increased but the polyunsaturated fatty acids (PUFAs) decreased. Hernández-Hernandez et al. [140] showed that the application of biostimulants based on algae and amino acids supplemented with nutrients on olive plants from the *cvs* 'Chemlali' and 'Arbequina' (in high density orchards) improved fruit weight but not alter oil quality (e.g., fatty acids, polyphenols and tocopherols). In olive orchards, the application of *A. nodosum* reduced olive oil acidity (%) but improved to levels of total phenols [4]. Almadi et al. [46] did not report changes in the characteristics of olive fruits (fresh weight, pigmentation, water content, oil content and yield efficiency) from the *cv* 'Leccino' treated with a protein hydrolysate (Sinergon 3000, CIFO, Bologna, Italy).

Concerning biostimulant effects on olives yield, Tejada et al. [141] reported an improvement with the use of two biostimulants obtained from sewage sludge by fermentative processes using *Bacillus licheniformis* (ATCC 21415) in olive tree from orchards.

5. Concluding Remarks

Changes in agriculture production must be implemented to meet the Farm to Fork agricultural strategy of the EU, adapt to climate change and promote agroecosystems biodiversity. Therefore, the search of innovative and sustainable strategies to the agricultural system has increased in the last decades. The use of biostimulants, that are natural products, microorganisms or even nanomaterials, emerge as a sustainable strategy to achieve the objective of developing a more sustainable agricultural production, reducing the irrigation water requirements and the needs of agrochemicals under the actual context of climate change. In olive culture, the use of biostimulants remains very limited with respect to other crops, such as grape. However, the promising results obtained particularly in the control of some important olive diseases, open good perspectives for their application to other disease as well as to improve olive performance and productivity under abiotic stress conditions (like drought and heat). These results also pinpoint that more research must be conducted to better exploit the benefits of biostimulants in olive groves, as a complementary approach to other sustainable practices and the valorisation of more tolerant cultivars. Finally, the knowledge gained here may be useful in spreading the use of biostimulants to other economically important crops.

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