

The Use of Biostimulants in Sustainable Viticulture

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ABSTRACT: Viticulture has a very wide application area in the world, which is great importance economically and in terms of human diet. The grapevine is evaluated in many areas, both grapes and leaves contain valuable compounds such as vitamins, minerals, antioxidants, organic acids, fats, proteins etc.. It has become inevitable that sustainable production techniques, the use of new integrated techniques, and sufficient sensitivity to protect human and environmental health have to be applied for viticulture which is of great importance in terms of human diet. Sustainable viticulture includes human and environment friendly production systems. It is seen that biostimulants, in other words bioactivators, are used within the scope of scientific researches and in viticulture applications in sustainable viticulture in the world. Containing organic or inorganic compounds, microorganisms; biostimulants are applicable to leaves, soil or seeds; positively affect plant growth, yield, nutrition, and product quality. It has been determined by various studies that biostimulants increase the resistance of plants to biotic and abiotic stress conditions and also regulates the soil structure. Biostimulants have been classified by some researchers as humic substances, amino acids and other nitrogenous compounds, seaweed and plant extracts, chitin and chitosan-like polymers, inorganic compounds, beneficial fungi and beneficial bacteria, waste, exudates and extracts of seeds, leaves and roots. Biostimulants have an important place within the scope of sustainable viticulture in areas such as protection of natural resources, especially soil and water, combating erosion and forest fires, ensuring biological diversity, and integrated pest management. The need to increase soil and plant productivity, to create ecological balance, and most importantly to protect the health of humans and other living things, is better seen each day. This need for a sustainable life and healthy continuity of future generations leads scientists and producers to friendly applications such as biostimulants.

Keywords: Biostimulants. sustainable production, viticulture

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INTRODUCTION

Sustainable agriculture is an agricultural structure where natural resources are protected in the long term and contains agricultural technologies do not harm the environment. In other words, sustainable agriculture is an approach that aims to balance environmental, social, ergonomic and economic dimensions in agricultural production. Sustainable agriculture aims to keep the economy alive in the short and long term, to increase the quality of life of the producers, and to develop application areas in this direction, while maintaining productivity on the one hand, and reducing the damage to the environment on the other (Turhan, 2005).

Sustainable viticulture aims to restore the disturbed natural balance as a result of faulty applications in the ecological system. It includes human and environment friendly production systems. It envisages organic and green fertilization, alternation, soil conservation, increasing plant resistance, benefiting from parasites and predators. Sustainable viticulture ensures that all these possibilities are created in a comprehensive system and is a production model that aims to increase the quality of the product rather than increase the amount in production (Anonymous, 2020a).

As in all agricultural fields, the importance and necessity of sustainability in viticulture is better understood every day. This situation has led researchers to investigate different plant activators in the field of viticulture.

Affecting plant growth, nutrition, product quality and yield positively; in order to increase the resistance of plants to abiotic stress. Biostimulants are materials that are applied to plants from the leaves, soil or seeds, which may contain organic or inorganic compounds, microorganisms, and some of them also have regulatory effects on the soil structure. Biostimulants are also called "Biological Stimulants" or "Plant Activators" (Du Jardin, 2015; Külahtaş and Çokuysal, 2016; Roupheal, 2018;).

As the trend towards healthy agricultural products has increased in recent years, it is observed that scientific researches using biostimulant products are increasing day by day and the product market is constantly expanding (Povero et al., 2016). France, Italy and Spain are the leading EU countries in commercial biostimulant production (Traon et al., 2014). Biostimulants are also recommended agronomic tools to counter abiotic stress. These products, which contain bioactive molecules, have a beneficial effect on plants and combat adverse environmental conditions by affecting primary or secondary metabolism. Plant protection products that only have a direct effect against biotic stresses should not be included in the biostimulant category. For a plant activator to be called a biostimulant, the product must also be effective against abiotic stress conditions on the plant (Bulgari et al., 2019).

Classification of Biostimulants

It is seen that different researchers show different approaches when classifying biostimulants. Especially in recent studies, researchers have listed different activators as biostimulants.

These categories are;

1. Humic substances
2. Amino acids and other nitrogenous compounds
3. Seaweed and plant extracts
4. Chitin and chitosan-like polymers
5. Inorganic compounds
6. Useful fungi and beneficial bacteria (Du Jardin, 2015).
7. Wastes (Yakhin et al., 2017).
8. Exudates and extracts of seeds, leaves and roots (Yakhin et al., 1998; Ertani et al., 2014; Yasmien et al., 2014; Lucini et al., 2015; Ugolini et al., 2015).

Humic substances

Humic substances are the natural organic substances found in soil, water, sediments of decayed animals and plants. In other words, humic substances are called macromolecular compounds formed during degradation of plants in soil or surface waters, which are natural organic materials found in the environment. Fulvic and humic acids are the most widely used bioactivators in agriculture.

1. Humin: It is a part of humic substances that cannot be soluble in water at any acid or alkaline pH.
2. Fulvic acid: It is a part of humic substances that are in water soluble form under all pH conditions.
3. Humic acids: The main content of organic substances in the soil is humus. Humic acid is the most active ingredient of humus. Humic acids are the main compounds obtained from soil (Anonymous, 2020b). They have undertaken tasks such as controlling the availability of plant nutrients, ensuring the Carbon and Oxygen exchange between the soil and the atmosphere, and ensuring the transformation of toxic chemicals into different components (Piccolo and Spiteller, 2003; Anonymous, 2020c).

The mechanism of action of humic substances; change the physical properties of the soil and increase its water holding capacity; affect the cation exchange capacity and buffering properties and nutrient availability of the soil; encourage the transformation of numerous elements into forms that plants can use; increase the permeability of plant membranes. Humic substances also stimulate the growth of various beneficial microorganism groups; encourage the root systems of plants to further development and cell division and expansion; increase enzyme (complex proteins that can accelerate the rate of chemical reactions) and hormone systems (Anonymous, 2019a).

The effects of humic acid applications on the yield, grape characteristics and nutrient uptake of Ercis grape cultivar (*V. vinifera* L.) in Van province of Turkey were examined during two growing seasons in 2003-2004 (Cangi, et al, 2006). Solid form of humic acid (55 % humic acid, 30 % fulvic acid and 8 % K₂O) were applied from soil in the amount of 100, 200, 300 kg ha⁻¹ and 10, 20, 30 kg ha⁻¹ from leaves. As a result, these researchers stated that SSC, total acid, N and Fe contents of leaves of grape vines were affected by humic acid application. In cool ecology such as Van province, increase in SSC by humic acid application will be contribute to ripening of grapes before autumn frost (Cangi, et al, 2006).

In a study conducted by Morard et al. (2011) on vineyards, it was observed that the taste of grape berries improved as a result of the humic acid applications they applied. In a study, the response of two grape varieties (Feteasca Regala and Riesling) by spraying three different concentrations of humic acid onto leaves was investigated in order to demonstrate sustainable viticulture practices to farmers. As a result of the research, it was observed that there was an increase in growth, yield and fruit quality in vines (Popescu and Popescu, 2018).

In a study in which cane steels belonging to the cv. Sultani Çekirdeksiz and Narince and cv. Erciș were used, in addition to determining the tolerance to salt, the biochemical contents and some physiological parameters were investigated in order to determine the protection mechanisms of the used Humic acid doses in the plant under stress conditions.

It has been observed that humic acid applications increase the resistance of the plant to salty soil conditions. When the treated plants and control plants were compared in terms of macro-microelements, it was observed that humic acid applications generally increased mineral substance intake, although the results differ according to the elements, application doses and plant parts (Ersayar, 2017).

Protein hydrolysates and amino acids

These biostimulants are obtained from both plant and animal industrial products. Plants can directly use amino acids and peptides to increase their development and resistance to stressors (Watson and Fowden, 1975; Ertani et al., 2009).

Protein hydrolysates function as plant growth regulators thanks to the peptides they contain (Ryan et al., 2002). Protein hydrolysates help plants to uptake more Nitrogen by promoting Carbon and Nitrogen metabolism in plants (Külahtaş and Çokuysal, 2016). Protein hydrolysates have direct effects on plants as well as indirect effects. When these products are used, microbial activity becomes more active in soils (Du Jardin, 2015).

The amino acids found in biostimulants can be included in the protein structure in plants as well as non-protein amino acids. In some studies, it has been observed that non-protein amino acids such as glutamate, histidine, proline, glycine and betaine are applied externally and these amino acids protect the plants from abiotic stress factors or stimulate the metabolic signaling mechanisms in plants (Sharma and Dietz, 2006; Forde and Lea, 2007).

Polat (2006) conducted a study to determine the effects of biostimulants on growth of grapesapling in 2006 by employing two different biostimulants (Agrozym and Almina) at four different dosages (0, 5, 10, 20 g per pot) in one year old grape sapling planted in 10 liter of pots in 2006 vegetation period. It was found that biostimulants, overall, caused positive effects of plant growth. While both biostimulants did not lead to any changes at budding dates, the shoot length and weight, chlorophyll level, root length and weight increased to a great extent. While macro plant nutrient of levels (N, P, K, Ca, Mg, and S) did not change, the micro nutrients (Mn, Fe, Zn, Mo, and B) increased greatly (Polat, 2006).

Biostimulants containing amino acids are supportive of plants' resistance to oxidative conditions and defense mechanisms against abiotic stressors. Glycine betaine and proline are osmotic regulators that stabilize amino acids, enzymes and cell membranes under high salt concentrations and temperature conditions that disrupt plant growth (Ashraf and Foolad, 2007, Dos Reis et al., 2012, Ahmad et al., 2013). In addition to being effective in salt and drought stress in plants, proline also provides resistance to plants in heavy metal stress and regulates plant growth by removing reactive oxygen species (Sharma and Dietz, 2006).

In order to increase the quality of a vineyard with good growing conditions, the eco-physiological and productive characteristics of the vine in the cv. Sangiovese were investigated. As a result of the research, it was found that the application of these biostimulants provides a balance during phenolic maturity, preserves the sugar content of fruits and increases the anthocyanin and polyphenol contents (Salvi et al., 2015).

Two protein hydrolysates from soybean (soy) and casein (casein) were tested to trigger the resistance of grapevines against downy mildew (*Plasmopara viticola*) pathogen, which has the most destructive effect on European and North American vineyards. It was determined that the infected grapevine leaf surface was reduced by 76% and 63%, respectively, with the application of soy and casein in cv. Marselan. In general, although the effect of soybeans is more pronounced than casein hydrolysate, both hydrolysates increased immunity in grapes against pathogen attack (Lachhab et al., 2014).

Seaweed extracts

The use of seaweed as organic matter and fertilizer goes back a long way in agriculture. However, the effects of biostimulant products in agriculture have just begun to be noticed. The fact that it contains polysaccharides, alginates, carrageenan and their by-products has enabled the use of seaweed in agriculture (Külahtaş and Çokuysal, 2016). In addition to the chelating properties of these extracts that

facilitate the intake of plant nutrients, they also positively affect the soil structure and aeration, and have a regulatory effect on plant growth and development (Milton, 1964).

Seaweed extracts are also considered as biostimulants because they improve important criteria such as seed germination, seed development, plant growth, yield, flower and fruit formation, resistance to biotic and abiotic stress factors, and post-harvest shelf life (Mancuso et al., 2006; Rayorath et al., 2008; Khan et al., 2009; Craigie, 2011).

In a study which is conducted in the vineyard, it was found that the root development increased as a result of the foliar application of seaweed extracts (Mancuso et al., 2006). This situation was associated with the increase in lateral root development and the increase in total root volume and length, with phytohormones such as auxin and cytokine found in seaweed extracts (Khan et al., 2011a, 2011b). At the same time, Kuwada et al. (1999) was concluded that root growth observed in plants in soils where moss extracts were applied positively affected microorganism activity in these soils.

The cv. Carménère grown in Chile has a serious impact on plant productivity due to its tendency to reproductive disorders. Since the cv. Carménère is an important variety for the region, the effect of seaweed extract, which is a plant growth regulator, on fertilization disorder has been investigated. In the research; some parameters such as the effect on fruit cluster and cluster characteristics, number and weight of fruits, cluster weight, grain size and distribution of clusters, and the physicochemical of grape must were measured. In the study, in addition to brown algae (*Ascophyllum nodosum*), boron ethanolamine and a lot of zinc oxide were given. The results showed that there was an increase in the number and weight of fruits per cluster in cv. Carménère. Therefore, it has been concluded that it is possible to improve reproductive disorders caused by excessive sun exposure by treating it with certain micro elements such as boron and zinc in addition to seaweed extract (Gutiérrez-Gamboa et al., 2018).

Chitin and chitosan biopolymers

These components are used in food, cosmetics, medical and agricultural sectors. Chitin and chitosan biopolymers are mostly obtained from marine products and mushrooms. For physiological effects of chitin and chitosan in plants, The positive effects of their ionic structure on the protection effect of DNA, plasma membrane, cell wall, cell parts and stress factors and related activation of genes have been observed in some studies (Hadwiger, 2013; Katiyar et al., 2015).

The positive effects of chitosan, such as protection from fungal pathogens, resistance to abiotic stressors and increasing the fruit quality are increasing day by day. It has been determined that chitosan applications have effects such as stomata closure due to abscisic acid in plants and the development of defense mechanism of this component against environmental stressors (Iriti et al., 2009).

Inorganic compounds

Inorganic compounds obtained from organic materials can also find use within the scope of sustainable agriculture. Inorganic compounds facilitate the growth and development of plants (Anonymous, 2019b). These components generally do not contain carbon, since they are inorganic, they are not synthesized in the living body but taken from the outside ready, they have a structure that can pass directly into the cell without being digested, and they mostly play regulatory roles in living things (Anonymous, 2020d).

Downy mildew disease in grapevines is one of the most important and destructive diseases in viticulture. Copper-based fungicides are used in organic vineyards to control disease. However, since 2006, the use of copper has been limited by a EU regulation. In the study, 112 different biostimulants and other methods, including biocontrol agents, were examined. Animal originated materials, homeopathic preparations, inorganic substances, microbial extracts, natural derivatives, plant extracts,

physical methods and synthetic materials were used in the study. While not as effective as copper for controlling downy mildew in organic vineyards, *Yucca schidigera* (a tree in the form of a bush) and *Salvia officinalis* (sage) extracts and *Trichoderma harzianum* (Biocontrol agent) could be cited as promising candidates. Potassium bicarbonate (Armicarb and Salukarb), which does not contain copper, was used as an inorganic substance in the study and it was found that it effectively reduced downy mildew infection in wine grapes. Armicarb, SaluKarb, Gro-stim, Kendal and Ulmasud were used among the inorganic substances. The purpose of this study is thought to help develop an integrated management program in which a less effective product is applied with copper to reduce the total amount of copper applied (Dagostin et al., 2011).

Inoculants

Microbial inoculants used in agriculture are divided into two groups as bio-pesticides and biological fertilizers. Bio-pesticides are a kind of pesticide, that are obtained from many natural materials in the form of animals, plants, bacteria and various minerals and are based on the use of vectors (harmful) in plant protection. They are less harmful compounds than chemical pesticides. While these directly affect the target pest and closely resembling creatures; conventional pesticides affect a wider group, including birds, insects and mammals (Yarsan and Çevik, 2007). Biological fertilizers are agricultural inputs that contain live microorganisms and can be applied to seeds, different surfaces of plants and soil. The most common biological fertilizers may include beneficial bacteria (PGPR= Plant Growth Promoting Rhizobacteria) some fungi, or mycorrhizal fungi. Especially biological fertilizers are included in the biostimulant group (Vessey, 2003).

The beneficial microorganisms PGPR's work as biostimulants by assuming tasks such as symbiotic N fixation, making plant nutrients useful, siderophore production, facilitating Fe uptake and production of volatile organic compounds (Çakmaçi, 2005).

Trichoderma isolate is among the important microorganisms due to their capacity to control phytopathogenic fungi. Some *Trichoderma* isolates have a significant biostimulant effect. It can be used in both solid and liquid form. *Trichoderma* spp. has a positive effects such as phyto-stimulation mechanism, increasing root, branching and nutrient intake capacity, communication with root and shoot systems, connection with the rhizosphere, and significant increase in plant growth and yield have been observed in many studies (López-Bucio et al., 2015).

In a study, different doses of *Bacillus subtilis* PGPR isolates and *Trichoderma harzianum* were applied to investigate the effects on young plant development. As a result, it has been determined that *Trichoderma harzianum* and *Bacillus subtilis* have positive effects on planting success and development of 2-year-old Syrah / 110R young plants (Korkutal et al., 2017a; Korkutal et al., 2017b).

In a study conducted in the field of vegetables, it was aimed to determine the combinations with different hybrid and standard tomato varieties and some PGPR bacteria and to examine the reactions of the best combinations under salt stress. In the first stage of the study, 3 hybrids and 3 standard tomatoes varieties were used as plant material. Eight bacterial species tested in previous studies were used as bacterial inoculum sources. It has been determined that PGPR isolates provide the positive effects on seedling development parameters in tomato plants under salt stress and facilitate the intake of nutrients such as K and Ca, which are important in nutrient content, especially in salt stress (Yilmaz, 2017).

In light of the work has been completed, the promotion of the use of particular viticulture area of PGPR isolate and widespread, without the need for inorganic plant nutrition and plant protection materials, it has been found to apply the most ideal form (Gazioglu Sensoy, et al., 2019).

Mycorrhizal fungi inoculates to the roots of many plants in the ecosystem through the spores existing in the soil. This situation increases the resistance of the plant to drought and some diseases. These fungi mycelium networks, known as the web of nature, can settle in the roots of plants as well as provide connections between plants that are far apart. They share nutrients with other plants, or they can release toxic chemicals to harm plants they do not like (Anonymous, 2019c).

In another study conducted in the vineyard, mycorrhiza and humic acid were applied to increase the quality characteristics of the seedlings in the production of grafted rooted tubular vine saplings. As a result of the application; The vegetative growth such as root number, root thickness, root length, shoot length, shoot thickness of seedlings, and the grafting success level have affected varying rates according to variety / rootstock combinations. As a result of the research, it has been determined that both humic acid and mycorrhiza applications significantly increase the seedling yield (Kavak, 2006). It seems that the effect of mycorrhiza applications in sustainable viticulture studies is promising.

Korkutal et al. (2020), use two different mycorrhiza cocktails (Symbion Vesicular Arbuscular Mycorrhiza, Shubhodaya Vesicular Arbuscular Mycorrhiza) were applied to the young grapevines four different methods (Control, Planting mixture, Root, Root + Planting mixture). Researchers were used cv. Alphonse Lavelleé and cv. Razakı as a plant material. The young grapevine performance, shoot, leaf and root characteristics were examined; leaf, shoot, and root mineral compounds were analyzed. As a result, it has been demonstrated that the most beneficial application to young grapevine in both cvs and both different mycorrhizal preparations is the application to the soil mixture.

Organic wastes

Food waste or industrial waste streams, composts and compost extracts, fertilizers, vermicompost, waste water and sewage treatment have been included in the biostimulant group by some researchers (Yakhin et al., 2017). Agricultural wastes are divided into three groups:

1-Wastes remaining as a result of plant production; the vegetative mass that comes out as a result of the crop production in the cultivated land, forest, fallow land, fruit and vegetable cultivated areas and cannot be qualified as a product is considered as waste. Stem, straw, buck, bark, seed, pruning residues can be included in this group.

2-Wastes remaining as a result of animal production; it is the internal organs left over as a result of animal manure and slaughter. Animal manures are used as fuel (dung) and as fertilizer. It is possible to use wastes from internal organs as compost fertilizers.

3-Wastes resulting from the processing of agricultural products; they are the wastes generated as a result of the processes (grinding, sorting, drying etc.) of agricultural products before they are directly used.

These are unused wastes such as stalk, straw, shell and seed. Knowing the effects of post-processing wastes of these products on soil properties is necessary for a useful and appropriate recycling. The pulp obtained after processing is called biochar. The wastes arising from grape products are evaluated in different areas: grape (peel, seed and grape waste) were used as growing medium, silage additive, in poultry feed, dye (for food or fabric), feed additive, in the cosmetics industry, in aromatherapy, and pruning residues are used as fuel. (Bekar, 2016).

Exudate and extracts of seeds, leaves, shoots and roots

This biostimulant group is obtained from the exudates of seeds, leaves and roots. For the most part, it is extracted from high plants such as *Amaryllidaceae* (daffodils), *Brassicaceae*, *Ericaceae* (terraceae), *Fabaceae* (legumes), *Fagaceae* (chestnuts), *Moringaceae*, *Plantaginaceae*, *Poaceae*, *Rosaceae*,

Solanacea, Theaceae, Vitaceae (Parrado et al., 2008; Pretorius, 2013). This biostimulant group gives positive results in the growth and development of the plant, its yield and quality, and in the fight against diseases, in sustainable viticulture (Parrado et al., 2008; Pretorius, 2013; Atıcı, 2016).

In a study investigating the possibility of using grape-shoot extracts as a biostimulant in viticulture, the extract obtained from the shoots of the same vine was applied to the cv. Airén leaves. In the resulting; it was determined that it positively affected the alcohol degree, pH, total acidity, volatile acidity, color density, variable aroma potential index, phenolic compounds and yield. These results are predicted to contribute to sustainable viticulture (Sánchez-Gómez et al., 2016).

CONCLUSION

Sustainable agriculture is an extremely important concept in terms of creating a society that meets its own needs without endangering future generations. Conservation of our natural resources, especially soil and water, combating soil erosion and forest fires, ensuring biological diversity, integrated medicine management, using organic production models and appropriate cultivation techniques, is of great importance for sustainable living.

While the regular and effective use of organic materials from different origins has a positive effect on some physical and chemical properties of the soil; at the same time, it provides significant support for a sustainable agricultural production without harming human beings and nature. Productivity-oriented conventional agricultural activities, which are carried out without considering human, animal and environmental health, destroy our soil and water resources over the years, and inorganic preparations that are used extensively sometimes cause irreversible problems. However, the return of natural products and natural wastes to nature will not cause negative reactions in the ecosystem, but will provide significant increases in productivity and quality. In addition to some applications sensitive to human and environment, we also benefit from the substances we call biostimulants; it will provide great advantages in terms of clean food, clean environment and healthy life. With the use of these substances, human and environmental health is protected, while operating costs can be minimized in terms of agricultural inputs.

The use of biostimulants has an important place in sustainable agriculture and sustainable viticulture practices. The use of humic substances, amino acids and other nitrogenous compounds, seaweed and plant extracts, chitin and chitosan-like polymers, inorganic compounds, beneficial fungi and beneficial bacteria, organic wastes, seeds, leaves and root exudates and extracts, without harming the ecology and human health and abiotic problems. It also has positive effects on the growth levels and bioactive components of plants.

If the grape, which is both a fruit with high table value and an industrial product, is grown with sustainable methods at the growing stage, the product obtained will of course be more valuable. Considering that consumer tendencies change in a positive way and the level of consciousness increases day by day; sustainable viticulture will increase the marketability of grapes grown with clean agricultural practices in the consumption of products obtained from fresh, dried or must. Especially the grape that turns into wine, which is a product with high economic value all over the world, will reach a higher market share with the use of biostimulants.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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