Review article
Journal of Central European Agriculture, 2025, 26(1), p.229-239
DOI: /10.5513/JCEA01/26.1.4396

Enhancing organic fruit production: insights into biostimulant applications for sustainable growth, quality, and resilience in diverse agroecological settings

Poboljšanje organske proizvodnje voća: uvid u primjenu biostimulansa za održivi rast, kvalitetu i otpornost u različitim agroekološkim okruženjima

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Received: May 12, 2024; accepted: January 12, 2025

ABSTRACT

This article explores the application of biostimulants in organic fruit production, focusing on apples, olives, citrus fruits, and stone fruits. Biostimulants, diverse substances comprising organic and inorganic compounds and microorganisms, play a crucial role in enhancing soil fertility, crop resilience, and pest control in sustainable organic farming. The article highlights the impact of various biostimulants on the growth, yield, and quality of apples. Noteworthy findings include the influence of marine macroalgae on leaf area, chlorophyll content, and fruit coloration. Additionally, biostimulants containing zinc and silicon prove to be effective in reducing physiological disorders during storage. For olives, the positive effects of biostimulants such as amino acids and organic acids on biochemical properties are discussed. Algal extracts and mycorrhizal fungi are explored for their role in enhancing olive resistance to stress conditions and controlling diseases like *Verticillium* wilt. In citrus fruits, the application of biostimulants mitigates the negative impacts of drought and soil salinity. Algal extracts, chitosan, and seaweed extracts demonstrate positive effects on vegetative growth, fruit set, and physiological activities in citrus plants. Stone fruit production, including peaches and cherries, benefits from biostimulants like humic and fulvic acids, beneficial bacterial strains, and silicon. These biostimulants improve the efficiency of nutrient utilization, increase resistance to diseases, and enhance fruit quality. In conclusion, the review underscores the potential of biostimulants to improve production efficiency, product quality, and plant resistance to abiotic stresses in organic fruit cultivation. However, further research on specific crops, varieties, and optimal application periods is emphasized to maximize biostimulant benefits in diverse agroecological conditions.

Keywords: fruit quality, humic acids, sustainable agriculture, seaweed extracts, stress resistance, yield

SAŽETAK

U radu je prikazana primjena biostimulansa u organskoj proizvodnji voća, s naglaskom na jabuke, masline, agrume i koštičavo voće. Biostimulansi u održivom organskom uzgoju sastoje se od organskih i anorganskih spojeva te mikroorganizama i imaju ključnu ulogu u poboljšanju plodnosti tla te povećanju otpornosti biljaka na napad štetnika. U ovom radu prikazan je utjecaj različitih biostimulansa u proizvodnji jabuka na rast, prinos i kvalitetu plodova. Značajna otkrića uključuju utjecaj morskih makroalgi na površinu lista, sadržaj klorofila i boju plodova. Biostimulansi koji sadrže cink i silicij pokazali su se učinkovitima u smanjenju fizioloških poremećaja tijekom skladištenja. Kod maslina su uočeni pozitivni učinci biostimulansa, poput aminokiselina i organskih kiselina, na biokemijska svojstva. Ekstrakti algi i mikoriznih gljiva istražuju se zbog njihove uloge u povećanju otpornosti masline na stresne uvjete te u kontroli bolesti poput verticilijskog venuća. Kod agruma, primjena biostimulansa ublažava negativne utjecaje suše i zaslanjenosti tla. Ekstrakti algi, hitosan i ekstrakti morskih algi pokazali su pozitivne učinke na vegetativni rast, zametanje plodova i fiziološke aktivnosti citrusa. U proizvodnji koštičavog voća, poput bresaka i trešanja, biostimulansi poput huminske i fulvinske kiseline, korisnih sojeva bakterija i silicija također imaju pozitivno djelovanje. Ovi biostimulansi poboljšavaju učinkovitost iskorištenja hranjivih tvari, povećavaju otpornost na bolesti te unaprjeđuju kvalitetu plodova. Zaključno, pregled naglašava potencijal biostimulansa u poboljšanju učinkovitosti proizvodnje, kvalitete proizvoda i otpornosti biljaka na abiotske stresove u organskom uzgoju voća. Međutim, ističe se potreba za daljnjim istraživanjima specifičnih biljnih vrsta, sorti i optimalnih razdoblja primjene kako bi se maksimizirale koristi biostimulansa u različitim agroekološkim uvjetima.

Ključne riječi: kvaliteta voća, huminske kiseline, održiva poljoprivreda, ekstrakti morskih algi, otpornost na stres, prinos

INTRODUCTION

One of the main aims of organic farming is to sustainably manage soil fertility, increase plant resilience and combat pests and diseases in an environmentally friendly way. The use of biostimulants is an effective tool for achieving these goals in agriculture. Biostimulants consist of various organic and inorganic substances and microorganisms. Depending on their origin and composition, they can be divided into humic substances and complex organic substances (protein hydrolysates, amino acids, humic and fulvic acids), useful chemical elements, microbial inoculants (arbuscular-vesicular mycorrhiza, N-fixing bacteria) and algal extracts (Calvo et al., 2014). Biostimulants are a relatively new category of agricultural preparations and substances, so there is not yet a uniform definition.

Moreover, the definition of plant biostimulants is often controversial, as several organic or inorganic compounds from different sources have a stimulatory effect on plants (Rodrigues et al., 2020). In addition, it is important to understand that biostimulants do not act as fertilizers, pesticides, or repellents. It is considered that the effect of biostimulants is indirect. One of the definitions states that biostimulants are any substance or microorganism applied to plants, seeds, or roots to stimulate natural processes in plants that promote the efficiency of nutrient use and/or tolerance to abiotic stress, regardless of the content of nutrients and/or microorganisms intended for that purpose (Traon et al., 2014). Unlike fertilizers or phytohormones, biostimulants promote and stimulate the life processes of plants. Their influence on plants does not derive from a direct ability to regulate metabolism and its effects can be multidirectional. Crucially, unlike bioregulators and hormones, biostimulants enhance plant metabolic processes without altering their natural processes (Posmyk et al., 2016; Jurić et al., 2021). Biostimulants can be easily substituted by bioregulators, fertilizers, or biopesticides, but there are differences in the mechanism of action, origin, active ingredients, the concentration at which they are applied, etc. Compared to plant growth regulators, biostimulants have a wider range of sources and more diverse functions. Plant biostimulants affect not only plants but also the soil and soil microorganisms.

Biostimulants used in fruit growing to increase growth, yield and quality are often easy to apply and can be used in different ways. Spraying and irrigation are two of the methods available for applying biostimulants. In foliar spraying, the nutrients contained in the product are absorbed by diffusion through the leaf cuticle (Taiz and Zeiger 2014). Fertilisation, on the other hand, involves the application of diluted biostimulants in water that seeps into the soil, with uptake by the roots predominating. However, this method is less used than foliar application (de Goes et al., 2021). However, depending on the type of product, the active ingredients and the crop grown, there are certain ideal and recommended ways of using each biostimulant (Vinceković et al., 2019; Jurić et al., 2020). The biological effects of biostimulants often depend on the cultivated species and are strongly influenced by external conditions, i.e., growth conditions (Andreotti et al., 2022). During application, the method, dosage, and timing of application are particularly important. Preparations based on algal extracts, humic and fulvic acids are applied mainly as foliar, while protein hydrolysates and beneficial chemical elements are applied mainly to soil (by soaking) or alternately, foliar (Andreotti et al. 2022). Although some biostimulants were used in agriculture before the development of synthetic fertilisers and pesticides, the development of biostimulants has improved in the last 10 years and has thus become a new and promising technology in organic agriculture.

The effect of biostimulants is perceived through the following agronomic parameters, such as the increase in yield, the accumulation of biomass and the improvement of fruit quality (Fraznoni et al., 2022) through the accumulation of secondary metabolites, mainly phenolic components. Plant secondary metabolites are a heterogeneous group of molecules, with phenolic compounds representing the largest group of secondary metabolites in plants. Phenolic compounds play a key role in the tolerance of plants to abiotic stress factors due to their high antioxidant potential.

On the biochemical side, biostimulants activate certain enzymes in plants, activate the antioxidant defence mechanism and lead to more efficient assimilation of nutrients (Fraznoni et al., 2022). Changes in photosynthesis, chlorophyll concentration and transpiration are observed as a physiological reaction in plants (Fraznoni et al., 2022).

Genetic variability, synergistic/antagonistic effects of some biostimulants and environmental factors that influence the efficacy of biostimulants are facts that make determining the mode of action of the biostimulants themselves even more complex.

This means that experiments need to be carried out under protected conditions and outdoors on model plants such as *Arabidopsis thaliana* or the tomato variety Micro-tom and other non-model species to simulate different environmental conditions. For this reason, standardised protocols are needed to evaluate the mode of action of biostimulants on crops (Franzoni et. al, 2022).

In recent years, omics sciences such as transcriptomics, proteomics and metabolomics have helped to improve the understanding of the mechanisms underlying the action of plant biostimulants by identifying the molecular and biochemical pathways involved and revealing their regulatory role in metabolic pathways. These techniques make it possible to obtain information useful for clarifying the molecular basis of the phenological changes induced by the application of biostimulants (Xu et.al, 2022).

Many preparations contain high concentrations of organic substances such as enzymes, proteins, vitamins, plant hormones, carbohydrates, pigments, phenolic compounds, and antimicrobial compounds. These can act directly on the cultivated fruit trees and interfere with the mechanisms of natural defence reactions, plant metabolism, gene regulation, growth and yield. They can act indirectly in the soil by forming organic acids, improving microbial activity and the amount of organic matter, which consequently reduces the consequences of unfavourable conditions and increases plant resistance. The use of biostimulants has proven to be particularly effective and desirable for crops that are frequently exposed to temperature, drought, and salt stress. Organic fruit production, like other areas of agriculture, is confronted with the consequences of climate change and anthropogenic activities, which refer to the frequent and unpredictable occurrence of abiotic and biotic stresses. Drought, soil salinity, lack of availability of nutrients in the soil, soil degradation, introduction of resistant pests and temperature stress are just some of the problems faced by fruit growers, causing major yield and quality losses in fruit production. Therefore, the use of biostimulants can be an important tool to improve production. However, addressing certain limitations and overcoming challenges is essential for maximizing the potential benefits of these approaches (Table 1).

But despite the many limitations and challenges, the use of biostimulants is a first-class innovation with great potential to improve sustainable crop production.

As a cutting-edge technology, the use of biostimulants in many fruit crops has not yet been researched. Most scientific work relates to the most commercialised crops (apples, olives, citrus, cherries, etc.) and even in these, not all groups of biostimulants have been thoroughly researched. In a review, Basili et al. (2020) provided an overview of research on the effects of biostimulants on fruit crops exposed to abiotic stress factors such as drought, salinity and temperature and did not focus on organic production. It is extremely interesting to show the effects of biostimulants precisely in this production system, especially in improving crop productivity, which is one of the shortcomings of the organic production system. Some of the studies mentioned in the paper did not specify whether the orchard was organic, but the overall effect of biostimulants applies to both organic and conventional fruit production.

Table 1. Key limitations and challenges associated with the use of biostimulants in organic fruit production

Limitations	Challenges	References
Consistency and standardization	Biostimulants are derived from diverse sources and have varied compositions, leading to inconsistencies in their quality and efficacy. The lack of standardized methods for evaluation and comparison of their effects may challenge farmers in choosing the most effective products.	Saporta et al., 2019
Understanding mechanisms of action and research gaps	Mechanisms through which different biostimulants affect plant growth and stress resistance are yet to be fully understood. The complexity of the plant–biostimulant–abiotic stress relationship remains a complex gray area that needs to be defined holistically. This lack of detailed knowledge may limit the effective application and optimization of these products.	Nephali et al., 2020; Jurić et al., 2020
Environmental impact	While generally considered environmentally friendly, the production and widespread use of biostimulants could have unforeseen environmental impacts. The over-extraction extraction of raw materials required for producing biostimulants could affect ecosystems.	Bulgari et al., 2019; Kapoore et al., 2021
Regulatory and approval processes	The regulatory framework for biostimulants is still evolving. There is a need for a legal and harmonised definition of biostimulants. In many regions of the world, there is a lack of clear regulatory guidelines, which can hinder the development and approval of new biostimulant products.	Du Jardin et al., 2015
Farmer knowledge and training	There is often a knowledge gap among farmers regarding the optimal use of biostimulants. Incorrect application can lead to reduced efficacy or unintended negative effects.	Yakhin et al., 2017; Fleming et al., 2019
Adaptation to local conditions	The effectiveness of biostimulants can vary significantly depending on local soil types, climate conditions, and specific crop varieties. Tailoring biostimulant use to these local conditions remains a challenge.	Aamir et al., 2020; Jurić et al., 2020
Compatibility with other agricultural inputs	Understanding how biostimulants interact with other agricultural inputs like fertilizers, pesticides, and soil amendments is crucial. Incompatibilities could reduce the effectiveness of these products or even harm the crops.	Jurić et al., 2020; Sani and Yong, 2022
Economic considerations, market access and distribution	The affordability of biostimulants can present a significant barrier, particularly for small-scale farmers who may find the expenses associated with these products to be a substantial financial burden. The economic return on investment for these products is not always clear, which can discourage their adoption. Additionally, the availability of specific biostimulants can vary greatly by region, affecting their accessibility to farmers. The distribution networks for biostimulants are less developed and less extensive compared to those for traditional agricultural inputs, which can limit their accessibility and availability to farmers.	Xu and Geelen, 2018; Crtichley et al., 2021

APPLICATION OF BIOSTIMULANTS IN APPLE PRODUCTION

Organic apple production in the world and the EU accounts for a significant share of total organic fruit production. The share of organic total EU apple production is estimated in the EU in the year 2023 to be around six percent, which is well below the 25% goal that the EU has set itself for 2030, making it highly unlikely that this goal will be achieved (USDA, 2023). In 2022 in Croatia, 779 t of apples from organically grown orchards were harvested (EUROSTAT, 2023a). Like other pome fruit species, apples require timely and detailed monitoring of the occurrence of diseases and pests, as well as protection that complies with organic farming regulations. Research on the application and effect of biostimulants on apples has shown the positive effect of different products on the growth, yield, resistance, and quality of apples. The research by Soppelsa et al. (2018) was conducted in an organic apple orchard on the 'Red Jonathan' variety by treating with 10 different biostimulants (humic acids, macro- and microalgae extracts, alfalfa protein hydrolysate, amino acids alone or in combination with zinc, B-group vitamins, chitosan and a commercial product containing silicon), for two years at weekly intervals since 40 days after full flowering (at the end of May) and until the end of August. The application of marine macroalgae (*Ascophyllum nodosum*) extract caused an increase in average leaf area of about 20%. A product based on zinc and macroalgae caused an increase in the relative chlorophyll content in the leaves, and the application of biostimulants based on B-group vitamins, chitosan and silicon resulted in higher photosynthetic rates and leaf transpiration compared to the control. The use of biostimulants did not influence the total yield in any year of the study. Algal extract (*Ascophyllum nodosum*), alfalfa protein hydrolysate and B group vitamins caused apple fruits to have the most intense red fruit colour and colour index values that were significantly higher than those of the control. Treatments with an extract of marine macroalgae *Ascophyllum nodosum* and microalgae *Spirulina* spp. and a biostimulant based on amino acids and zinc significantly increased the total phenolic content in the fruit skin. Treatments with alfalfa protein hydrolysate, macro - and microalgae, B-group vitamins and chitosan resulted in a double concentration of anthocyanin compared to the control. Biostimulants based on chitosan and silicon caused a higher antioxidant potential in the apple pulp. Marine macroalgae and microalgae extract, chitosan and silicon significantly reduced the incidence of Jonathan's spot disorders during different phases of cold storage. Apples treated with zinc showed a significantly lower incidence (about 60%) of physiological disorders of the fruits at the end of the storage period (4 months) as compared to the control. Soppelsa et al. (2020) continued to investigate the influence of biostimulants on apple fruit quality at harvest and after storage. Their results address the problem of calcium deficiency at the fruit level, which is one of the main causes of postharvest disorders in apples. For example, bitter pit, frequent apple postharvest disorder, is associated with calcium deficiency (Jemrić et al., 2016). The most common solution to increase the Ca concentration in apples is foliar application of Ca in the form of calcium chloride. In their study, apple trees in an organic orchard were treated with calcium chloride in combination with commercial biostimulant products containing zinc and silicon or algae extract (Soppelsa et al., 2020). The results showed that the application of marine algae increased the number of fruits and the final yield of apple trees prone to alternative bearing. (i) The silicon-based biostimulant significantly increased the number of fruits and yield per tree compared to the single treatment with calcium chloride. (ii) The marine algae extract affected the metabolism of plant hormones (mainly cytokines and abscisic acid), leading to the induction of anthocyanin biosynthesis and accumulation in the fruit skin before harvest. (iii) The combined application of calcium chloride with algal extract or with a zinc-containing product was effective in reducing the incidence of physiological disorders in stored apples. Furthermore, the higher concentration of Ca, Zn and Mn found in healthy apple fruit compared to fruit with defects (insect or psychological damage) may be evidence of the positive role of these elements in increasing the resistance of fruit to storage disorders. Di-Vaio et al.

(2021) studied the effects of biostimulants (microalgae, protein hydrolysates and algae extract mixed with zinc and potassium) on an 8-year-old apple orchard of the 'Annurca' variety in Vitulazio, Italy. Biostimulants based on protein hydrolysates and microalgae can be used to increase 'Annurca' production (10.41%), fruit diameter, percentage of first harvest (37.84%) and yield value (6.9%). The application of microalgae products favored an increase in the cross-section of the stem, while the seaweed-based products improved the vegetative activity of the plant. The application of biostimulants had no significant effect on the quality parameters of the fruit (firmness, total soluble solids (TSS), titratable acidity (TA) and pH).

APPLICATION OF BIOSTIMULANTS IN OLIVES PRODUCTION

The olive (*Olea europaea* L.) is a fruit plant whose production accounts for a significant share of organic fruit production in Croatia and the rest of the EU. In 2022, 2294 tonnes of 40000 tonnes of olives harvested in Croatia were organically grown (EUROSTAT, 2023a). In addition, 1940 ha of olives were grown organically in Croatia in 2020 (EUROSTAT, 2023b). The olive tree is known to be a robust and adaptable species that thrives even on poor Mediterranean soils. The environmental conditions in olive groves are often extreme and olive growers have to deal with stress problems caused by drought and salt. Biostimulants, as substances and products suitable for such conditions, can provide an ecologically sound solution to achieve higher yield and quality of organically grown olives and olive oil. Research results of Nargesi et al. (2022) have shown that foliar application of biostimulants on three olive cultivars (Zard', Arbequina', and 'Manzanilla') based on amino acids and organic acids had a positive effect on the biochemical properties of olive fruits. The trees treated with arginine and humic acid had the highest protein content in the fruits as well as the total chlorophyll content, and the trees treated with humic acid had the highest content of anthocyanins and phenols. Similarly, the influence of the applicated biostimulants was found to be cultivar related. Also, algal seaweed extract (Kelpak, *Ecklonia maxima*) applied at a dose of 6 mL/L foliar increased leaf surface area, leaf chlorophyll content, leaf dry weight and leaf carbohydrate content (Al-Hadethi, 2019). In addition, studies on the effect of algae-derived polysaccharides (ulvan, alginate and laminarin) on olives showed interesting results in controlling olive wilt caused by the fungal pathogen *Verticillium dahliae*. Polysaccharides from algae stimulated phenol metabolism and the results showed a reduction in wilt symptoms, and it is concluded that polysaccharides from algae can help control olive wilt by strengthening host defence metabolism and limiting pathogen growth (Salah et al., 2018). Young olive seedlings, especially certain varieties, may be susceptible to fungal infection of the roots. Mycorrhizal fungi can also be used as biostimulants to protect plants. Mycorrhizal fungi are more competitive than pathogenic fungi in finding infection sites on the root surface, i.e., mycorrhizal fungi prevent pathogenic infection due to the ease of root infection. Moreover, comparing two olive cultivars both colonised by mycorrhizal fungi, Khalil and El-Ansary (2020) reported a positive effect of arbuscular mycorrhizal fungi on the increase of chlorophyll content in leaves, proline accumulation, growth index, total carbohydrates, and catalase activity. Mycorisation of seedlings or fruit trees is particularly important in areas with poor soil quality where water resources are limited. Mediterranean fruit crops (olives, citrus, kiwi, pomegranate, fig, almond, pistachio, carob, wine and other stone and pome fruits) in such areas require additional inoculation with mycorrhizal fungi to achieve greater plant resistance to biotic and abiotic stress, increased uptake of P and Zn, better root development of seedlings in depleted soils, and increased yield and fruit quality.

APPLICATION OF BIOSTIMULANTS IN CITRUS FRUIT PRODUCTION

Citrus fruit crops (oranges, lemons, mandarins, grapefruits, limes, etc.) are mainly cultivated in the Mediterranean and southern areas. Apart from the fact that agro-ecological growing conditions in the Mediterranean region are often extreme and unfavorable, climate change and negative anthropogenic influences further complicate the ecological cultivation of citrus fruits. The yield of citrus plants is often affected by drought stress and extreme temperatures. Under such conditions, the biochemical, metabolic, molecular, and physiological processes are altered, leading to growth retardation and economically significant yield losses. Therefore, the application of biostimulants as ecologically acceptable, available, and versatile products is considered one of the solutions to improve organic citrus production.

The algal extract of *Ascophyllum nodosum* applied foliar or by soaking successfully mitigates the negative effects of water deficit in young orange trees (cv. The sweet orange 'Hamlin' grafted on 'Carrizo' citrange and 'Swingle' citrumelo rootstocks) exposed to two different water regimes: 50% and 100% evapotranspiration (Spann and Little, 2011). Trees treated with algal extract showed higher vegetative growth - longer shoots, larger leaf area and higher dry mass of shoots and leaves. Microbial biostimulants based on arbuscular mycorrhizal fungi were also found to promote citrus resistance to drought stress (Wu et al., 2013). An earlier study by Wu et al. (2011) indicated that the tolerance of *Citrus tangerine* Hort. ex Tanaka to soil salinity was improved by seedling mycorrhization. Sweet orange cultivar 'Washington' foliar treated with chitosan (2 g/L) had a higher percentage of fruit set and yield, higher chlorophyll content and improved fruit physicochemical properties (Mohamed and Ahmed, 2019). Ali and Araji (2020) indicated that foliar treatment of lemons with seaweed extract resulted in an increase in the number of leaves and their surface area. They attribute this result to the cytokinin contained in the extract, which stimulates the physiological activities of the plant and increases the chlorophyll content in the leaves, which has a positive effect on photosynthesis and carbohydrate composition. In addition, they found that algae extracts change the hormonal balance of plants and thus influence biological processes that improve metabolism, which in turn promotes the vegetative growth of the plant.

APPLICATION OF BIOSTIMULANTS IN STONE FRUIT PRODUCTION

As with other fruit crops, organic cultivation of stone fruit often encounters problems and challenges such as abiotic and biotic stresses, which consequently affect lower yield and economic losses for farmers. Studies show that the use of biostimulants, a relatively new technology in agriculture, can be a good solution to the problems mentioned. When it comes to the efficiency of nutrient use, biostimulants are suitable and effective tools in several studies. For example, the application of humic and fulvic acids in the soil has been reported to significantly increase the concentration of zinc and iron in '0900-Ziraat' sweet cherry trees (*Prunus avium* L.) grafted on Gisela 6 rootstocks. This is particularly important for cultivation on calcareous soils where stone fruit species are often sensitive to iron chlorosis (Abay and Pirlak, 2017). In a study (Arıkan et al., 2018) conducted on peach trees, root inoculation with beneficial bacterial strains resulted in a significant increase in the concentration of active iron in leaves., while study of Saa et al. (2015) with foliar application of *Ascophyllum nodosum* algal extract resulted in stronger vegetative growth of almond trees grown under conditions with insufficient or satisfactory availability of potassium in the soil. The authors conclude that a biostimulants based on the extract of the above-mentioned seaweed can significantly influence the increase in potassium uptake under conditions of potassium deficiency in the soil (Arıkan et al., 2018). Silicon belongs to the group of microelements, i.e., to a group of minerals whose daily requirement is between 1 mg and 100 mg. Although silicon occurs in larger quantities in the soil, it is difficult for plants to access because it is released only very slowly from clay minerals. Similarly, silicon is effective against cherry cracking due to its ability to limit the water permeability of the cell wall and provide greater elasticity to fruit tissue (Soppelsa et al., 2018). Gonçalves et al. (2020) and Correia et al. (2019) reported that *Ascophyllum nodosum* algal extracts in sweet cherry have reduced fruit cracking, reduced cell membrane damage in leaves, improved water status, increased soluble sugar, organic acid and carotenoid content, higher yield. The content of bioactive compounds, polyphenols, and vitamin C has also increased. The use of the brown macroalga *Ecklonia maxima* as a substrate for biostimulant use in cherries resulted in increased fruit set and yield (Gonçalves et al., 2020).

APPLICATION OF BIOSTIMULANTS IN NUT FRUIT PRODUCTION

The cultivation of nut fruit trees is becoming increasingly important worldwide due to their high nutritional value (Miljković, 2018). This is particularly true in Croatia, where the cultivation of hazelnut (*Corylus avellana* L.) and walnut (*Juglans regia* L.) has experienced a sudden increase in the last decade. In Croatia, walnuts and hazelnuts were cultivated on 8,420 and 6,710 hectares, respectively, in 2021, compared to only 2,978 and 2,649 hectares in 2013 (FAOSTAT, 2023). These two nut species also account for the majority of organically farmed orchards in Croatia. According to Grgić et al. (2020), walnuts make up 42.4% and hazelnuts 24.3%. Considering the fact that nowadays more attention is paid worldwide to the health, safety and quality of nuts (Vrtodušić et al., 2022), and due to the limited availability of organically grown products, biostimulants can prove to be very useful in nut production. In addition, as climate change progresses and extreme weather conditions become more frequent, biostimulants can be used to mitigate their negative effects. Pascoalino et al. (2021) investigated the effect of different biostimulants on hazelnut 'Ennis' and almond (*Prunus dulcis* (Mill.) D.A. Webb) 'Vairo'. Four different biostimulants (phytoalgae - an algal extract from *Ascophyllum nodosum*; foliar B - boron as ethanolamine, 15.4% w/v; amino acids - free amino acids, 28.8% w/v; soil B - sodium tetraborate pentahydrate, 14.85% B) were tested on almonds and five on hazelnuts, two of which (phytoalgae and amino acids) are compatible with organic farming principles. In almonds, the application of phytoalgae significantly increased the total tocopherol content, while leaf and soil B reduced it. In hazelnuts, the same effect was observed for amino acids (which had no significant effect in almonds), while phytoalgae only slightly increased their content on average. Tocopherol, a class of fat-soluble compounds with vitamin E activity, is best known for its antioxidant effect (Duncan and Chang, 2012) and therefore this effect can be considered positive. However, Pascoalino et al. (2021) also reported a significant reduction in the fat content of almonds and hazelnuts treated with phytoalgae, as well as almonds treated with leaves. Kelting et al. (1997) investigated the effect of humate-based biostimulants on Turkish hazel (*Corylus colurna* L.) seedlings. They concluded that no treatment increased the growth of the tree crown compared to untreated trees. However, it should be noted that some practices were carried out during the experiment that are not compatible with the principles of organic farming, but because humate-based biostimulants can be used in organic farming, this study was considered. Simon et al. (2023) investigated the responses of walnut 'Alsószentiváni 117' to foliar applications of various biostimulants (Wuxal Ascofol - algal extracts from the seaweed *Ascophyllum nodosum*; Kondisol - humic and fulvic acids of different sizes, plus enzymes, co-enzymes, polysaccharides, various macro-, micro- and meso-elements; Alga K Plus - a foliar fertiliser with a very high content of potassium (K_2O 30%) and algae). In 2020, the weight of dried nuts was significantly lower in the control trees than in the trees treated with Wuxal Ascofol, as was the weight of dried kernels compared to the treatments with Wuxal Ascofol and Alga K Plus. No average difference was observed in 2021. It is also worth noting that although no significant difference was reported, the control trees had the conspicuously smallest nut length and diameter in 2020. Regarding the biochemical analysis, the authors reported that the walnuts treated with Wuxal Ascofol and Alga K Plus had a significantly higher catechin content in 2020 than the control walnuts, while no significant difference was found in 2021. The content of chlorogenic acid and rutin was significantly higher in the Kondisol-treated walnuts in 2020, while no significant difference was found in 2021 compared to the control. The quercetin content was significantly higher in the kernels of walnuts treated with

Kondisol and Alga K Plus in 2021 than in the control and the other treatments, while no significant differences were found in 2020. Ellena et al. (2023) investigated the effect of seaweed extract (Stimplex, Acadian AgriTech, Nova Scotia, Canada) on hazelnut 'Tonda di Giffoni'. This treatment included two foliar applications (3 L/ha) every 15 days at the time of fruit set in late November and mid-January and one application of seaweed extract during fruit development in late January. Fruit yield per tree, kernel yield per tree, fruit weight and kernel weight were significantly higher, while the proportion of empty fruit was significantly lower in the treated hazelnuts. No significant difference was found with regard to the proportion of kernels.

CONCLUSIONS

Numerous studies have shown that biostimulants can improve production efficiency, quality, and product safety. Treatment of fruit crops with humic and fulvic acids, protein hydrolysates, amino acids, chitosan, beneficial chemical elements, algal extracts, and microbial biostimulants can significantly increase the efficiency of using other agronomic inputs such as irrigation water or fertilisers. The greatest benefit of using biostimulants is to promote the resistance of plants to various abiotic stresses and to improve the final quality of the product. The potential of biostimulants is recognised in the field of organic fruit production, but more research is needed on the efficacy of their use in field growing systems, under specific agroecological conditions, and in perennial cultivation. To improve and disseminate biostimulants, it is also necessary to study the effects of biostimulants on certain species and varieties that have not yet been researched, as well as the optimal period of application. Based on the available evidence, it can be stated with certainty that biostimulants represent an ecologically acceptable potential for integration into the management of modern organic orchards and can increase the sustainability of production and the efficiency of resource use.

Still, significant challenges remain in the field of biostimulants application. Ensuring consistency and standardisation in the use of biostimulants across varied agroecological settings is crucial for their effective implementation and reliable outcome measurement. Additionally, a deeper understanding of the mechanisms of action of biostimulants and addressing existing research gaps are essential. This knowledge will not only optimise their use but also minimise potential environmental impacts. Effective navigation through complex regulatory processes, coupled with improving farmer knowledge regarding biostimulant use, are also key areas that require further development to fully harness the benefits of biostimulants in sustainable agriculture.

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