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REVIEW

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Biostimulants research in some horticultural plant species—A review

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Abstract

Different substances from the natural origin which have beneficial effects on plant growth and development, stress resistance, and crop yield and quality can be called biostimulants or biostimulators. Their physiological effects depend on their composition as they contain various organic and mineral compounds which plants can use as metabolites, growth regulators, and nutrients; however, biostimulants cannot be considered biofertilizers. Biostimulants applied in plant production have been widely considered as an environment-friendly agricultural practice—and so are now among tools used in sustainable agriculture. Here, we discuss the results of the biostimulants' effect investigations performed in Croatia, focused on horticultural crops, with edible plant species, such as tomato, garlic, bell pepper, lettuce, strawberry, garden cress, and basil, as well as ornamentals, such as wild rose, wax begonia, Mexican and French marigold, moss rose, everlasting flower, common zinnia, English primrose, and scarlet sage. The investigated biostimulants were applied at all plant growth stages, from germination to full plant and fruit or flower commercial maturity, using the seed treatment, foliar application, or irrigation. To evaluate biostimulant effectiveness, various morphological, physiological, and quality traits were analyzed. In this wide array of studies, the evaluated biostimulants mostly enhanced seed and transplant vigor, stimulated vegetative growth, improved nutrient acquisition and distribution within the plant, increased antioxidative capacity of plant tissues, contributing to higher stress tolerance, and improved plant yield and fruit/flower quality. In general, the research reviewed here implies possible benefits of biostimulant application in horticultural production, especially in stressful growth conditions, such as the transplant stage, reduced fertilization, or incidence of other abiotic stress. Considering possible interactions among the contained physiologically active compounds, the effects on plants may depend on dose, time of treatment, growth conditions, and plant species. Therefore, further research of biostimulant applications in horticultural production is suggested.

KEYWORDS

biostimulants, fruits, horticulture, ornamentals, vegetables

1 | INTRODUCTION TO THE BIOSTIMULANTS APPLICATION IN PLANT PRODUCTION

1.1 | Background

There has been a plethora of research undertaken to identify functional amendments to be used in plant production to improve plant growth, productivity, and quality, as well as to help plants overcome different types of environmental stress. Nowadays, horticultural production has to cope with the increasing challenges of meeting high productivity with global demands for environment-friendly crop management practices. In organic agriculture, the use of chemical fertilizers and pesticides is limited (Pascual et al., 2018), so there is a need for different plant amendments suitable for such production. In the face of degraded agricultural areas and uncertainties related to the changing climate, biostimulants use can be an interesting option. Vernieri, Borghesi, Ferrante, and Magnani (2005) consider the combination of hydroponics and biostimulants as a promising environment-friendly production strategy for vegetables grown in greenhouses. According to Traon, Laurence, Ferdinand, and Du Jardin (2014), "A plant biostimulant is any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants benefiting nutrient use efficiency and/or tolerance to abiotic stress, regardless of its nutrients content, or any combination of such substances and/or microorganisms intended for this use." Yakhin, Lubyanov, Yakhin, and Brown (2017) defined biostimulants as "a formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds."

Depending on their composition and expected results, biostimulants can be soil- or leaf-applied (Kunicki, Grabowska, Sękara, & Wojciechowska, 2010). In the last two decades, special attention has been given to compounds of plant origin and to some other natural bioactive materials, such as humic and fulvic acids (Calvo, Nelson, & Kloepper, 2014; Canellas et al., 2015; Nardi, Pizzeghello, Schiavon, & Ertani, 2016; Shah et al., 2018). Their physiological effects occur after their entrance into plant tissues and cells, where these compounds are involved in the plant's metabolism, signaling, and hormonal regulation of growth and development.

Among diverse biological amendments, Abbott et al. (2018) pointed out biostimulants based on amino acids, chitosan, seaweed extracts, and humic substances. Seaweed extracts are the source of diverse compounds—such as lipids, proteins, carbohydrates, phytohormones, amino acids, osmoprotectants, antimicrobial compounds, and minerals—which can have biostimulating effects when applied as plant growth supplements. The use of seaweed extracts as biostimulants was thoroughly reviewed by Khan et al. (2009), Craigie (2011), Sharma, Fleming, Selby, Rao, and Martin (2014), Battacharyya, Babgohari, Rathor, and Prithiviraj (2015), and Nabti, Jha, and Hartmann (2017). Among horticultural crops, it has been shown that seaweed extract treatment improved seed vigor of bean (Carvalho, Castro, Novembre, & Chamma, 2013) and elicited proline accumulation in bean leaves under severe drought stress (Carvalho, Castro, Gaziola, & Azevedo, 2018). Definitions of biostimulants frequently mention protein hydrolysates (Colla et al., 2015; De Pascale, Rouphael, & Colla, 2017; Nardi et al., 2016) and microbial inoculants (De Pascale et al., 2017), which both can contribute to plant stress resistance and can boost nutrient uptake and distribution within the plant.

In addition to antistress supporting, stimulating growth, enhancing nutrient absorption, and increasing crop productivity, biostimulants also help break dormancy, increase fruit size, enhance root system development, increase the activities of photosynthetic and other vegetative tissues, enhance plant vigor and uniformity, regulate flowering, and stimulate fruit set and ripening. All these effects add up to the improved and balanced growth, development, and productivity of crops. Farmers, investors, regulators, consumers, scientists, and other interested players are still learning about biostimulants and their role in sustainable agriculture (EBIC, 2018). It is still an open question as to how growing conditions influence a biostimulant's uptake and biological activity in the plant. Another question still to be answered is to what extent an established biostimulant effect can be modified by a genotype-specific plant response at cellular and molecular levels. As an example, humic substances and other biostimulants related to plant hormonal activity may change the electrochemical gradient of protons formed across the cell membranes via modulation of proton pumps (Zandonadi et al., 2016). The eustress crop management using biostimulants or some other compounds of biotic origin may induce a balance between plant xenohormetic potential and crop yield. In order to do so, however, various elements of such management must be taken into account, such as dose, the period of application, specification of its duration, and plant age and developmental stage (Vargas-Hernandez et al., 2017).

As a precondition for understanding the effects of biostimulants on plants, the complex composition of commercially available products must be considered. Van Oosten, Pepe, De Pascale, Silletti, and Maggio (2017) stated that "such products had for a long time been loosely defined, and often dubiously regarded, because of their aggregate nature and the inherent difficulty to determine which specific components

TABLE 1 An overview of the research on biostimulants' effects on the growth, development, productivity, and quality of (a) edible horticultural crops in Croatia (b) ornamentals in Croatia

Plant species	Growth stage	Biostimulant	Evaluated parameters	Main conclusion	Source
(a)					
Capsicum annuum L.	After transplanting up to 7th harvest	Radifarm; Megafol; Benefit; Viva	Fruit yield per plant, largest fruit weight, smallest fruit weight, fruit number per plant;	Biostimulant application enhanced fruit yield parameters	Tkalec et al. (2010)
	After transplanting up to 8th harvest	Radifarm; Megafol	The antioxidative activity of pepper fruits; total and commercial yield, number of noncommercial, and BER-affected fruits	Increased commercial yield and antioxidative capacity in fruit	Parađiković et al. (2010a,b)
	Vegetative growth fruit bearing	Radifarm; Megafol	Leaf and fruit N, P, K, Ca, Mg, Fe, Mn, Zn concentration; BER incidence	Enhanced leaf and fruit macro- and micronutrient content; enhanced Ca concentration in fruit, a result linked to fewer BER-affected fruits	Parađiković et al. (2011a)
	5-7 leaves	Radifarm; Megafol; Viva; Benefit	Fruit total yield, average fruit weight, number of noncommercial fruits; leaf pigments content; total phenols, ascorbate, antioxidative activity in fruits	Increased yield; improved antioxidant activity, ascorbate, and phenolic content in fruits; increased pigment contents in leaves	Parađiković et al. (2011b)
	From transplanting to harvest	Radifarm; Megafol; Viva; Benefit	Yield, BER fruits	Increased yield; decreased number of BER-affected fruits	Parađiković et al. (2013)
Solanum lycopersicum	Transplants	Radifarm	Root length, fresh mass	Improved development of tomato transplant roots	Parađiković, Vinković, T., Teklić et al. (2008)
	Transplants	Radifarm; Megafol	Root, stem, and leaves fresh mass	Enhanced growth of tomato root, stem, and leaves; enhanced development after transplanting; improved plant adaptation to transplant stress	Vinković et al. (2009)
	Transplants	Radifarm; Megafol	Root, leaf, stalk – mass; nutrient content (N, P, K, Ca)	Enhanced uptake and distribution of mineral nutrients in plants	Vinković et al. (2010)
	Before and/or after transplanting	Radifarm; Megafol	Root, leaf, stalk—mass; nutrient content	Enhanced vegetative growth and increased mineral uptake	Vinković et al. (2012)
	Before and/or after transplanting	Radifarm; Megafol	Leaf area index, specific leaf area, leaf dry matter content	Stimulated leaf area development; increased leaf dry matter accumulation	Vinković et al. (2013)
	After transplanting till 2nd truss	Viva	Leaf: peroxidase and superoxide dismutase activity, pigments, protein content, phenols, antioxidative capacity; fruit: total soluble solids, total acidity, lycopene, ascorbic acid; yield	Decreased antioxidative enzyme activity under reduced NPK fertilization; affected neither yield nor fruit quality; biostimulant combined with reduced NPK fertilizers enabled stability of cell homeostasis in tomato plants and their better adaptation to stress	Koleška et al. (2017)

(Continues)

TABLE 1 (Continued)

Plant species	Growth stage	Biostimulant	Evaluated parameters	Main conclusion	Source
Lactuca sativa L.	After transplanting	Bio-algeen S-90	Plant height, leaf number, head mass, total yield, marketable and nonmarketable yield, chlorophyll, carotenoid and nitrate content, leaf juice pH, vitamin C, dry matter, N, P, K content	Positive effect on growth and yield parameters; enhanced vitamin C and dry matter content; decreased pH value; no effect on NPK content	Dudaš et al. (2016)
Lepidium sativum L.	Germination	KE-Plantasalva with and without sea salt; Fermentierter Pflanzenextrakt; Biplantol Universal; Acker-Schachtelhalm extrakt	Germination rate; seedling fresh and dry mass; root length	Plant extracts used as seed treatment enhanced water uptake and seedling development; all extracts increased germinability; Plantasalva with sea salt addition diminished seedling development	Lisjak et al. (2015)
Fragaria x ananassa Duch	Fruit bearing	Viva; Megafol	Leaf: antioxidative activity, ascorbate peroxidase, catalase, glutathione reductase, guaiacol peroxidase; fresh and dry trifoliate leaf mass, whole plant mass	Stimulated antioxidative enzymes in leaf under reduced NK fertilization; Megafol enhanced vegetative growth (fresh and dry mass)	Špoljarević et al. (2010)
	Flowering, fruit bearing	Viva; Kendal; Megafol	Fruit yield	Among tested biostimulants, viva improved fruit yield per plant	Stanisavljević et al. (2010)
	Fruit bearing	Viva; Megafol	Fruit yield; total antioxidant capacity; total ascorbic acid; total anthocyanins; total phenolics; nitrate content	Enhanced yield in spring cultivation; increased nutritional quality	Štolfa et al. (2013)
	Before flowering and until the start of fruit ripening	Porcine blood-based biostimulant	Number of fruits per plant, mean fruit weight, fruit yield per plant, the percentage of frozen flowers	Improved frost resistance and increased fruit weight; unclear effect on yield	Bogunovic et al. (2015)
Allium sativum L.	After transplanting	Radifarm	Total plant mass, bulb mass, number of cloves per bulb	Enhanced plant growth and development after transplanting	Parađiković et al. (2014)

(Continues)

TABLE 1 (Continued)

Plant species	Growth stage	Biostimulant	Evaluated parameters	Main conclusion	Source
Ocimum basilicum L.	Transplants	Radifarm	Plant height, number of leaves, root length; the fresh and dry mass of roots and above ground parts	Enhanced growth and development of roots and above ground parts	Zeljković et al. (2014)
(b)					
Begonia semperflorens L.	Transplants	Radifarm	Root and above ground fresh and dry mass	Improved growth and development of root and above ground mass	Zeljković et al. (2009)
	Transplants	Radifarm	Fresh and dry mass of roots and above ground plant parts; NPK content	Enhanced nutrient uptake in young plants and their growth and development	Zeljković et al. (2010b)
	Transplants	Radifarm	Fresh and dry mass of stem and root	Improved growth and development of root and above ground mass	Zeljković et al. (2011)
	After transplanting	Radifarm	Fresh and dry weights of roots and above ground plant parts; plant height; numbers of leaves and flowers; content of N, P, K, Ca, Mg; free proline content in roots	Increased all morphological parameters; stimulated nutrient uptake; increased free proline content in roots	Parađiković et al. (2017)
Rosa canina L.	Multiplication in tissue culture	Radifarm	Root mass and length; stalk mass	Increased root mass	Tkalec et al. (2012a)
	Multiplication in tissue culture	Radifarm	Number of shoots, stem height, root length, stem and root mass	Increased shoot number and root weight; improved growth and development of root and above ground mass of transplants	Tkalec et al. (2012b)
	After transplanting	Radifarm	Root length and mass	Enhanced plant growth and development after transplanting	Parađiković et al. (2014)
Tagetes erecta L.	Germination	Radifarm	Germination energy, germination rate; seedlings fresh matter content and dry matter content	Increased germination energy and seedling fresh matter content	Parađiković, Vinković, & Radman (2008)
	Transplants	Radifarm	Above ground fresh and dry mass	Improved growth and development of root and above ground mass	Parađiković et al. (2009)
Tagetes patula L.	Transplants	Radifarm	Root and above ground fresh and dry mass; NPK content in plant dry matter	Improved growth and development of root and above ground mass; enhanced nutrient uptake	Zeljković et al. (2010c)
	Transplants	Radifarm	Fresh and dry mass of stem and root	Improved growth and development of root and above ground mass	Zeljković et al. (2011)
	Transplanting and the next two months	Radifarm	Roots and above ground part weight; number of leaves, flowers and buds; plant height	Stimulated plant growth; increased number of leaves, flowers, and buds	Zeljković et al. (2013)
	Plantlets	Bio-algeen S-92	Plant height, leaf number, floral bud and open flower number	No effect on growth and quality parameters	Dudaš and Šestan (2014)

(Continues)

TABLE 1 (Continued)

Plant species	Growth stage	Biostimulant	Evaluated parameters	Main conclusion	Source
Salvia splendens L.	Transplants	Radifarm	Fresh and dry mass of roots and above ground plant parts	Improved growth and development of root and above ground mass	Zeljković et al. (2010a)
	Transplants	Radifarm	Fresh and dry mass of stem and root	Improved growth and development of root and above ground mass	Zeljković et al. (2011)
Primula acaulis L.	Transplants	Radifarm	Fresh and dry mass of stem and root	Improved growth and development of root and above ground mass	Zeljković et al. (2011)
Portulaca grandiflora *Helichrysum bracteatum* *Zinnia elegans*	Germination	Radifarm	Germination energy, germination; seedlings fresh matter content and dry matter content	Increased germination energy and seedling fresh matter content	Parađiković, Vinković, & Radman (2008)

Note. An overview of the research on biostimulants' effects on the growth, development, productivity, and quality of edible horticultural crops in Croatia.

were making beneficial contributions." Also, depending on the type of plant extract, the concentration applied, and the influence of many environmental factors, biostimulants may elicit different responses in treated plants (Lisjak et al., 2015). Abbott et al. (2018) pointed out the need for information that would enable farmers to discriminate among products with different levels of effectiveness; on-farm participatory research should contribute to addressing this need. Povero, Mejia, Di Tommaso, Piaggesi, and Warrior (2016) proposed an integrated approach based on chemistry, biology, and omics to understand the specific mode(s) of action of bioactive ingredients, an approach that should be taken to discover, evaluate, and validate potential new biostimulants.

1.2 | Biostimulants research in Croatia

Recently, several reviews collected the knowledge on plant biostimulant definitions, categories, modes of action in plants, and perspectives for further research and applications in agriculture (e.g., Brown & Saa, 2015; Bulgari, Cocetta, Trivellini, Vernieri, & Ferrante, 2015; Calvo et al., 2014; De Pascale et al., 2017; Du Jardin, 2015; Halpern et al., 2015; Nardi et al., 2016; Posmyk & Szafrańska, 2016; Rafiee et al., 2016; Tanou, Ziogas, & Molassiotis, 2017; Yakhin et al., 2017). We thus did not aim to make another comprehensive review of this kind, but instead to review the biostimulant research in Croatia, focusing on horticultural plant species. Commercial products declared as biostimulants were not available in Croatia for years and have appeared at the Croatian market not long ago. It is no wonder, then, that plant producers were unfamiliar with the possible benefits of biostimulants and only recently have started to learn about them.

This research (summarized in Tables 1 a and b) was conducted in Croatia and abroad, with the contribution of Croatian researchers. Biostimulant treatments were applied at various plant growth stages, from germination to full plant and fruit/flower commercial maturity, by means of seed treatment, foliar application, or irrigation throughout the vegetation. The applied biostimulants were mostly commercial products (Table 2). Among the investigated plant species were dietary relevant vegetables, fruit crops, and herbs, as well as many ornamental species. Croatia has a long tradition of horticultural production, but the country's producers—mostly small-scale—usually lack adequate financing to modernize their operations. Biostimulants may contribute to better plant productivity and quality, so small-scale producers need adequate information on their effects in particular crops. Thus, we present a wide array of Croatian research on commercial biostimulants in protected areas, such as glasshouses, plastic tunnels, and growth chambers, as well as in an open field, and compare these results with similar research elsewhere.

TABLE 2 The main functional components of the particular commercial biostimulants in the present review

Biostimulant	Active ingredients
Radifarm	Amino acids, glycosides, saponins, betaines, polysaccharides, organic acids, vitamins, microelements
Megafol	Amino acids (glycin, glutamic acid), betaines, proteins, vitamins (B5, PP, B1, B6), auxin, gibberellin, cytokine
Viva	Folic acid, vitamins (B6 and PP), polysaccharides, humic acids, proteins, peptides, amino acids
Benefit	Amino acids, nucleotides, free enzymatic proteins, vitamins
Kendal	Urea, oligosaccharides, glutathione, protein hydrolysate, saponins
Bio-algeen S-92	Seaweed *Ascophyllum nodosum* (L.) Le Jolis extract
Ruter AA	Free amino acids, macro- and microelements
Ergonfill	Animal protein hydrolysates, cysteine, folic acid, keratin derivatives
Asahi SL (Atonik)	Sodium para-nitrophenolate (0.3%), sodium ortho-nitrophenolate (0.2%), and sodium 5-nitroguaiacolate (0.1%)
Biozyme	Algae *Ascophyllum nodosum* extract, plant hormones (GA3+IAA+zeatin), chelated micronutrients
Goëmar BM 86	Algae *Ascophyllum nodosum* extract
Tablet	*Rhizophagus intraradices* and *Trychoderma atroviride* spores
KE-Plantasalva	Bio-molasses, herbs extracts, with or without sea salt
Fermented plant extract (Fermentierter Pflanzenextrakt)	Sugar cane molasses, lactic acid and photosynthetic bacteria, yeasts, grasses, weeds, garlic and chili pepper extracts
Biplantol Universal	Macro- and microelements, Ge, uronic acids
Equisetum extract (Acker-Schachtelhalm extrakt)	Flavonoids, plant acids, glycosides, Si
Algreen	Seaweed extract (*Sargassum* sp., *Ascophyllum nodosum*, *Laminaria* sp.), plant hormones, vitamins, free amino acids, alginic acid
Grow-plex SP	Liquid humate
Roots 2	A mixture of seaweed, humic acid and vitamins
Root & Shoot Builder	Seaweed extract (*Ascophyllum nodosum*), micronutrients, amino acids, natural chelating agents
Bio Rhizotonic	Algae extract, vitamins
Root Juice	Seaweed extract, humic and fulvic acids
Bio Root	Plant and mineral-derived organic acids and humates, alfalfa and soybean meal, brewers yeast, K-sulfate, rock phosphate, sea kelp
Slavol	Nitrogen-fixing and phosphate-mineralizing bacteria, auxins
Tytanit	Titanium ascorbate

Arioli, Mattner, and Winberg (2015) discussed the emerging Australian research of biostimulants based on seaweed extracts. Such reviews can broaden the general knowledge on biostimulant effects on crop plants and help foster international collaboration in further research on this topic.

2 | BIOSTIMULANT EFFECTS ON EDIBLE HORTICULTURAL SPECIES

2.1 | Bell pepper (*Capsicum annuum* L.)

Pepper (*Capsicum annuum* L.) is an important agricultural crop, not only for the economic value of its production but also for the nutritional value of its fruit. Indeed, pepper fruit is a rich source of vitamins (A and C), phenolics, carotenoids, and other health-related secondary metabolites with antioxidative function, such as capsaicin. Aminifard, Aroiee, Nemati, Majid, and Jaafar (2012) treated pepper plants with the fulvic acid solution and observed increased fruit antioxidant activity, total phenolics, carbohydrate, and carotenoid contents in fruit; total flavonoid and ascorbate content were not affected. In the research with two pepper cultivars treated with biostimulants, Parađiković, Vinković, Vinković Vrček, Žuntar et al. (2011; Table 1a) observed an increased antioxidative capacity in fruit, due to higher contents of vitamin C and phenols.

Biostimulants also contain different antioxidants or elicitors for their synthesis in plants, which may help plants to cope with unfavorable growth conditions, such as temperature extremes, limited water supply, and the resulting lower

nutrient uptake. Pepper plants treated with biostimulants Radifarm® and Megafol® showed higher antioxidative activity (Parađiković et al., 2010a,b); fruit yield increased up to 55%, and the incidence of unmarketable fruits affected with blossom-end rot (BER) decreased (Parađiković, Vinković, Vinković Vrček, Tkalec, et al., 2011). Similar results were obtained in another experiment with two bell pepper cultivars treated with biostimulants (Radifarm®, Megafol®, Viva®, and Benefit®; Table 2), where an exceptional influence of biostimulants on pepper yield was observed in a hot summer season, when high temperatures in a greenhouse caused physiological stress in plants (Parađiković, Vinković, Vinković Vrček, & Tkalec, 2013). Plants were grown hydroponically in rockwool slabs, and biostimulants were applied according to the providers' recommendation, mixed with the nutrient solution and applied by spraying or watering. Again, biostimulant application increased Ca content in fruit, number of fruits per plant, and mean fruit weight; the incidence of BER-affected fruits decreased. Radifarm® and Megafol® treatments affected the mineral composition of both vegetative organs and pepper fruit in plants grown in greenhouse conditions (Parađiković et al., 2013), but the two tested pepper cultivars responded differently to the biostimulant treatment.

The combination of Radifarm®, Megafol®, Benefit®, and Viva® increased fruit size of pepper plants grown under glasshouse conditions in cocopeat (Tkalec, Vinković, Baličević, & Parađiković, 2010). The effect occurred especially in the first harvest, indicating a better adaptation of the treated pepper plants to transplant stress. Since the taste, color, and nutrient qualities of pepper fruit can also depend on the content of antioxidant compounds (Parađiković, Vinković, Vinković Vrček, Žuntar et al., 2011), further insights into the factors which can affect their composition should help clarify which factors are important for the desired quality of peppers. According to the authors, phytochemical changes that occur during maturation and the resultant effect on antioxidant activity are important dietary considerations that may affect the consumption of different pepper types, provided that conscious consumers learn about these aspects of pepper quality. In Ertani et al. (2014), two plant-derived biostimulants (from alfalfa and red grape) increased the antioxidative activity and the contents of chlorogenic acid, p-hydroxybenzoic acid, and p-coumaric acid in green fruits of *Capsicum chinensis* L., while red fruits were highly enriched with capsaicin.

2.2 | Tomato (*Solanum lycopersicum*)

Tomato (*Solanum lycopersicum*) belongs to the most common vegetable crops whose production starts with nursery-grown plantlets from seeds in protected environments with optimal conditions for germination and early growth. Biostimulants such as Radifarm® may help tomato roots develop during plantlet growth, especially when growing conditions at this stage are not optimal for young plants (Parađiković, Vinković, Teklić, Guberac, & Milaković, 2008; Table 1a). By definition, a transplant is a seedling or sprouted vegetative propagation material grown in a substrate or in a field which is supposed to be transferred to the final cropping site (Pascual et al., 2018). After this process, the transplants enter a very sensitive growth period—they have to adapt to new environmental conditions, which may slow down their growth and development. This stressful stage of transplanting can be crucial for final plant productivity, depending on a stress level and a cultivar's genetic potential for stress tolerance. Transplants with a functioning root system can better adapt to new environmental conditions; being able to efficiently absorb water and nutrients, they can also grow faster and survive transplantation and after transplantation at a higher rate. Tomato plantlets treated before and/or after transplantation with Radifarm®, Megafol®, and their combination developed vegetative organs better than did control plants (Vinković et al., 2009). Radifarm® positively affected root growth while Megafol® stimulated the development of leaves (Vinković, Parađiković, Teklić, Tkalec, & Josipović, 2013; Vinković, Parađiković, Tkalec, Teklić, & Lončarić, 2012; Vinković et al., 2010). Burić, Čivić, Đurić, and Murtić (2012) also reported positive effects of three biostimulants (Bio-algeen S-92, Ruter AA, and Ergonfill; Table 2) applied after transplantation on the vegetative growth of tomato plants. All this suggests the benefits of the biostimulant application in a nursery production of tomato transplants.

Biostimulants also seem to enhance nutrient uptake in plants, but the underlying mechanisms of this effect of particular biostimulants are still unclear. Vinković et al. (2012) realized that tomato plants treated with biostimulants had greater contents of N, P, K, Ca, and Mg in plant dry matter than control plants did. Biostimulant application, however, was more efficient when applied before transplanting: Compared to the after-transplant treatment, it gave plantlets with higher stress tolerance, shorter adaptation to growing conditions in the glasshouse or in the field, and better fertilizer use efficiency. Positive effects of Radifarm® and Megafol® on plants' nitrogen status might be due to amino acids in the composition of these biostimulants (Table 2). Similarly, some other biostimulants based on protein hydrolysates can supply plants with amino acids which can affect plant metabolism, triggering auxin-like and gibberellin-like effects and enhancing nitrogen uptake and crop performance (Colla, Rouphael, Canaguier, Svecova, & Cardarelli, 2014; Vinković et al., 2012).

Better nutrient use efficiency due to the application of biostimulants makes them interesting for sustainable and environment-friendly plant production, mainly thanks to reduced fertilization, particularly of nitrogen. Koleška et al. (2017) studied the foliar application of biostimulant Viva® in tomato plants exposed to reduced NPK fertilization. The

biostimulant application along with reduced fertilization affected neither fruit quality nor yield, but it decreased the activity of superoxide dismutase and peroxidase in leaves. Sánchez Sánchez, Oliver, Cerdán, Juárez, and Sánchez-Andreu (2009) applied humic acids extracted and purified from a commercial product obtained from lignites to Fe-deficient young tomato plants grown in hydroponics. The biostimulative effect of humic acids on Fe uptake mechanism in plants led to alleviated symptoms of Fe deficiency as well as improved plant growth and Fe nutrition. Also, the tolerance of tomato to stress conditions can be improved through the application of biostimulants during field cultivation (Grabowska et al., 2015). The biostimulants the authors tested (Asahi SL, Biozyme, and Goëmar BM 86; Table 2) showed positive effects for most analyzed quality parameters of tomato.

2.3 | Lettuce (*Lactuca sativa* L.)

Lettuce (*Lactuca sativa* L.) is one of the most common leaf vegetables grown throughout the world in both greenhouses and open fields. Dudaš et al. (2016) stated that lettuce production in winter on the Adriatic coast, especially in nonheated plastic tunnels, requires longer cultivation and is characterized by lower head mass and yield. The main barriers for lettuce cultivation in this area are heat and water shortage in summer and the deficiency of light and frost incidence in winter. The authors investigated the effects of biostimulant Bio-algeen S-90 (Table 2), derived from the brown seaweed *Ascophyllum nodosum* (L.) Le Jolis, on butterhead lettuce growth, yield, and quality (Table 1a). The applied biostimulant had a better effect on plant height, leaf number, head mass, vitamin C, and dry matter content in lettuce leaves than did the applied fertilizer Megagreen (Ca fertilizer with micronutrients for foliar application). Both treatments enhanced plant growth and increased total yield, and both decreased nitrate content in leaves and the share of nonmarketable yield. The authors suggested an investigation of Adriatic algae extracts as possible biostimulants.

Colla and Rouphael (2015) consider biostimulant application as a sustainable tool for plant production and a meaningful approach to counteract salt and alkaline stresses. Accordingly, Rouphael, Cardarelli, Bonini, and Colla (2017) investigated the effects of Tablet (a biostimulant containing *Rhizophagus intraradices* and *Trychoderma atroviride*; Table 2), protein hydrolysates (PH) derived from legume seed, and their combined treatment on lettuce grown in saline or alkaline conditions. The application of biostimulant + PH was more effective than the microbial biostimulant application alone in protecting lettuce from stress conditions: The treated plants had better root system architecture, improved chlorophyll synthesis, and increased proline accumulation than control (untreated) plants did.

Conclusively, the application of biostimulants in lettuce growing may help in attaining better lettuce nutritional quality and help plants to overcome possible stressful growing conditions.

2.4 | Garden cress (*Lepidium sativum* L.)

Seedlings of garden cress (*Lepidium sativum* L.) have a spicy, tangy flavor. When consumed raw, they can be a valuable source of vitamins and other antioxidants. Malar et al. (2014) analyzed different parts of cress plants and concluded that shoot extract has the highest amount of ascorbate. Lisjak et al. (2015) tested five commercial biostimulants applied as seed treatment during germination (Table 1a). All the applied biostimulants enhanced germinability in comparison with the control variant, the greatest effect observed for fermented plant extract (Table 2), which increased germination by over 20%. KE-Plantasalva (Table 2) with sea salt addition actually had a negative effect, leading to the most pronounced root growth inhibition, probably due to the moderate salt stress it imposed on germinating seeds. The highest shoot fresh weight was obtained with KE-Plantasalva without sea salt, so this biostimulant could be recommended for application in cress seedling production. Among the biostimulants tested, Equisetum extract (Acker-Schachtelhalm extrakt; Table 2) most effectively stimulated dry matter accumulation in cress seedlings. This extract might have a large amount of silicone, whose positive effect on plant growth and development has been proved in various studies (Lisjak et al., 2015 and the citations therein).

2.5 | Strawberry (*Fragaria* × *ananassa* Duch.)

A popular horticultural crop for its attractive and appetizing fruit, strawberry (*Fragaria* × *ananassa* Duch.), offers much more than just delicious taste: Its fruit is valuable in human diet due to high amounts of antioxidants, mainly phenols and vitamin C. Correia et al. (2011) stated that within each cultivar, vegetative growth patterns might play an important role in the development of fruit quality traits—and so the research on biostimulants, which are well known to affect plant growth and development, has aimed to discover whether they can improve strawberry fruit quality. Stanisavljević et al. (2010) treated Diamante and Albion, ever-bearing and day-neutral strawberry cultivars, with biostimulants Kendal®, Megafol®, and Viva®, in field production conditions (Table 1a). Viva® had increased fruit yield while Megafol® and Kendal® did not, probably due to optimal growing conditions throughout the season and adequate fertilization applied by a drip irrigation system. Under reduced fertilization, however, the combination of biostimulants Megafol® and Viva® did increase

fruit yield of glasshouse-grown cultivar Elsanta (Štolfa et al., 2013).

Nutrient deficiency may trigger oxidative stress in plants, so Špoljarević et al. (2010) investigated antioxidative response in strawberry leaves to N and K supply and biostimulant application (Megafol® and Viva®) during the fruit-bearing period. Fertilization level interacted with biostimulant treatment. Lower N and K supply enhanced the activities of guaiacol peroxidase and catalase in the leaf tissues, while ascorbate peroxidase and glutathione reductase activities were stimulated in Megafol®-treated plants. Also, Megafol® treatment resulted in the highest leaf fresh and dry mass and vegetative plant mass.

According to Bogunovic, Duralija, Gadze, and Kisic (2015), the most dangerous condition during strawberry field production is late spring frost occurring when plants emerge from dormancy. In such a situation, biostimulants can support plant growth. In their study, all investigated strawberry cultivars treated with a biostimulant containing amino acids of animal origin had greater frost resistance, and the increased biostimulant concentration increased fruit weight. Plant growth stimulators with seaweed extract as one of the components increased marketable yield and fruit size but did not affect fruit juice pH, titratable acidity, and total soluble solids concentration (Roussos, Denaxa, & Damvakaris, 2009). Despite enhanced total anthocyanin concentration, the biostimulants affected neither fruit organic acid and carbohydrate concentration nor fruit color. Algreen—seaweed extract containing vitamins, free amino acids, alginates, and natural hormones (Table 2)—enhanced plant growth as well as fruit yield and quality of strawberry cultivar Sweet Charlie grown in field conditions (El-Miniawy, Ragab, Youssef, & Metwally, 2014). It did not, however, influence chlorophyll and nitrogen contents in leaves and ascorbate content in fruit. These varying—though never negative—effects of various biostimulants on strawberry yield and quality imply the need for further investigation of their modes of action as well as optimal cultivation practices, such as dosage and timing.

2.6 | Garlic (*Allium sativum* L.)

After onion, garlic (*Allium sativum* L.) is a second vital *Allium* species cultivated worldwide. Its bulb yield and quality vary with climate, cultural practices, and variety (Abdel-Razzak & El-Sharkawy, 2013). Parađiković, Tkalec, Zeljković, and Vinković (2014) applied biostimulant Radifarm® in the concentration of 0.25% immediately after the transplantation of regenerated plants produced *in vitro* into containers filled with commercial substrate and each following week until harvest (Table 1a). The authors compared total plant mass, bulb mass, and the number of cloves per bulb in treated and control plants. Since the biostimulant supplementation resulted in increasing all these traits, the authors concluded that biostimulant application could be considered good practice in garlic production, especially for overcoming stress during transplantation and for improving plant growth and development after transplantation.

Treatments with humic acids enhanced garlic bulb yield, bulb and clove diameter, and—by diminishing bulb weight loss—postharvest quality (Abdel-Razzak & El-Sharkawy, 2013). According to the available data on biostimulant composition (Table 2), Radifarm® does not contain humic acids as Viva® does, but all its other functional components might act synergistically to stimulate growth.

2.7 | Basil (*Ocimum basilicum* L.)

Basil (*Ocimum basilicum* L.) is an annual herbaceous plant from *Lamiaceae* family. Roshanpour, Darzi, and Haj Seyed Hadi (2014) consider it as one of the most important vegetable, spice, and medicinal plants. It is its aroma that determines the organoleptic quality and the medicinal value of basil (Ghazijahani, Hadavi, & Jeong, 2014). In general, biostimulant effects on growth, development, and quality of basil have been seldom studied. Kwiatkowski and Juszczak (2011) reported better growth, lower weed infestation, and an increase in herb yield of sweet basil plants (22%–31%) in the research with three growth stimulators (Asahi SL, Bio-algeen and Tytanit; Table 2). Similarly, Zeljković, Parađiković, Šušak, and Tkalec (2014) treated basil plantlets, after transplanting, with Radifarm®. The treatment enhanced the growth and development of roots and above ground parts (i.e., it increased the number of leaves, root length, and fresh and dry masses of root and above ground parts), thereby helping plants to adapt to transplantation stress (Table 1a); only plant height remained unaffected.

3 | BIOSTIMULANT EFFECTS ON ORNAMENTALS

3.1 | Wax begonia (*Begonia semperflorens* Link. et Otto)

Wax begonia (*Begonia semperflorens*) is an annual ornamental plant commonly grown in urban parks and private gardens, adapted to grow in both full sun and semi-shade. Low temperatures early in the season may lead to decreased nutrient uptake and plant growth, making it difficult to achieve the sufficient quality of begonia transplants for early outdoor planting (Parađiković et al., 2017). In many horticultural crops, transplantation stress may occur even in favorable growth conditions, until the root system of plantlets adapts to a new environment and starts supplying the plants with adequate water and nutrients amounts.

Biostimulant Radifarm® showed the potential of fostering root growth, transplant acclimatization, and further plant growth and development (Zeljković, Parađiković, & Oljača, 2009; Zeljković, Parađiković, Tkalec et al., 2010; Zeljković, Parađiković, Vinković, & Tkalec, 2011; Parađiković et al., 2017; Table 1b). Treated begonia plants had better nutrient uptake, higher free proline content in roots, higher plant fresh and dry weight, and more leaves and flowers per plant (Figure 1). Gibbons, Smaley, and Armitage (1996), however, did not observe any effects of three other biostimulants (Grow-plex, Roots 2, and Root n' Shoot; Table 2) on the growth of begonia transplants.

3.2 | Wild rose (*Rosa canina* L.)

Rosa sp. is usually propagated by vegetative multiplication methods such as cutting, layering, grafting, and tissue culture (Shirdel, Motallebi-Azar, & Mahna, 2012). Appreciated for its medicinal properties, dog rose or wild rose (*Rosa canina* L.) is also a useful rootstock for grafting and breeding cultivated rose species (Parađiković et al., 2014). Tissue culture enables faster reproduction of wild rose's rootstocks, but after the successful propagation of in vitro seedlings, it can happen that only 50% of seedlings survive the adaptation phase. The authors studied whether biostimulant Radifarm® could help

FIGURE 1 The effect of biostimulator (Radifarm®) on the root and shoot development of wax begonia (*Begonia semperflorens* Link. et Otto) plantlets (photo N. Parađiković)

overcome this problem. The biostimulant was first applied immediately after the transplantation of regenerated plants from tissue culture medium to pots containing commercial substrate and then once a week. The treated transplants had a 14% greater root mass than the control plants did. Tkalec, Parađiković, Zeljković, and Vinković (2012a,b) reported a significantly higher number of shoots in transplants treated with Radifarm®. Biostimulants enhanced also root development of wild rose transplants, helping them adapt to the new environment (Tkalec et al., 2012a; Parađiković et al., 2014; Table 1b). Since root formation after transplantation of young plants is a precondition of successful plant growth and development, rhizogenesis stimulation by hormone treatment is commonly applied in nurseries, during cutting production of many horticultural plant species.

Monder, Niedzielski, and Woliński (2014) and Monder and Pacholczak (2017) evaluated the potential of biostimulants with hormone-like activity for improving the viability of shrub rose cuttings. Shrub roses of older origin have a wide range of applications and are valuable for the preservation of biodiversity and historical heritage (Monder & Pacholczak, 2017). Monder et al. (2014) tested three environment-friendly biostimulants based on seaweed extract (Bio Rhizotonic, Root Juice, and Bio Roots; Table 2) and concluded that their use in optimal concentrations increased rooting ability and chlorophyll and protein contents in rose shoots. Monder and Pacholczak (2017) studied whether the phenological phases of rose stock plants and the above-mentioned biostimulants influenced the rhizogenesis of cuttings and changes in the content of polyphenolic acids. The content of polyphenolic acids correlated with rooting percentage. The results led the authors to conclude that biostimulants applied in *Rosa* sp. may help plantlet roots develop, whether produced in tissue culture or from cuttings.

3.3 | Marigold (*Tagetes erecta* and *Tagetes patula* L.)

Mexican marigold or Aztec marigold (*Tagetes erecta*) is an ornamental plant species with known medicinal use due to the high content of carotenoids in flower petals. Parađiković, Vinković, Teklić, et al. (2008; Table 1b) evaluated the effect of Radifarm® on seed germinability in several plant species, including *T. erecta*. Two concentrations of Radifarm® solutions were used for soaking germination paper. Among the investigated ornamental species, *T. erecta* seeds showed the highest germination rate in the optimal conditions of the germinator and the highest seedling fresh weight under the treatment with a higher Radifarm® concentration (0.5%). Applied additionally after the transplantation of Mexican marigold seedlings to substrate in the pots, the biostimulant increased above ground plant weight and root weight (Parađiković et al., 2009).

French marigold (*Tagetes patula* L.) is another ornamental plant of the genus *Tagetes*, in some countries also used to produce essential oil and as a spice. It belongs to horticultural plants commonly produced from transplants in greenhouses (Zeljković et al., 2013). As was with *T. erecta*, Radifarm® enhanced the growth and development of *T. patula* L. transplants (Zeljković et al., 2011). Applied as a single treatment after transplantation, the biostimulant increased nitrogen and potassium uptake in French marigold plantlets, resulting in their better growth and development than that of untreated plantlets (Zeljković, Parađiković, Vinković, Oljača, & Tkalec, 2010). In another experiment with Radifarm® application after transplanting, the biostimulant enhanced growth parameters such as fresh and dry weights of roots and above ground parts, number of leaves, nutrient status of plants, and flowering (Zeljković et al., 2013). In the second year of the experiment, under mild temperature stress due to low night temperatures, leaves of treated plants had higher proline concentration in plant tissue than those of untreated plants, implying a stimulating effect of Radifarm® on marigold stress tolerance.

Murtić et al. (2015), on the other hand, consider marigolds as undemanding plants which are able to bloom satisfactorily without biostimulation. In optimal conditions in their study, biostimulants Bio-algeen S-92, Slavol, and Ergonfill (Table 2) sprayed twice after transplanting increased the concentrations of photosynthetic pigments in marigold leaves. The biostimulants, however, affected neither flowering rate nor flower diameter. Similarly, Dudaš and Šestan (2014) applied Bio-algeen S-92 twice after transplanting marigold seedlings and observed no stimulating effect on plantlet growth and quality indices.

3.4 | Scarlet sage (*Salvia splendens* L.)

Scarlet sage or tropical sage (*Salvia splendens* L.) is one of the most popular ornamentals used to decorate public areas and home gardens (Zeljković, Parađiković, Babić et al., 2010). Like *Begonia semperflorens* L. and *Tagetes patula* L., *S. splendens* L. increased plantlet fresh and dry weight of roots and above ground parts under Radifarm® treatment at the transplant stage (Zeljković et al., 2011; Table 1b).

Jelačić, Beatović, and Lakić (2007) tested two biostimulants (Megafol® and Viva®) in the transplant production of *S. officinalis* L., another Salvia species. Viva® had a stronger influence on plantlet height, the number of branches, and plant fresh and dry weight. Viva® contains humic acids (Table 2), which might have improved the plantlets' nutrient uptake and thereby stimulated their growth.

3.5 | Primrose (*Primula acaulis* L.)

A Radifarm® solution applied after transplanting primrose (*Primula acaulis* L.) increased both root mass and fresh and dry matter content in the above ground parts of plants, the latter effect being stronger (Zeljković et al., 2011; Table 1b). The biostimulant did not affect *P. acaulis* root dry weight. Pumisacho

Gualoto (2015) tested the influence of different plant growing substrates, fertilizer/biostimulant made from several fruits: herbs extract (slurry), and one commercial seaweed-containing biostimulant, on primrose growth and flower productivity. The best results considering plant diameter determined 75 days after transplantation were achieved with herbs slurry, while the highest number of flowers per plant gave the treatment with fruit-based fertilizer/biostimulant. In conclusion, it seems that garden primrose (*P. accaulis*) reacts positively on different organic amendments with biostimulant effects.

3.6 | Moss rose (*Portulaca grandiflora*), strawflower (*Helichrysum bracteatum*), and zinnia (*Zinnia elegans*)

As mentioned above, biostimulant Radifarm® applied to seeds increased germination rate and seedling weight in *Tagetes erecta* (Parađiković, Vinković, & Radman, 2008; Table 1b). The authors applied the same treatment to seeds of *Portulaca grandiflora*, *Helichrysum bracteatum*, and *Zinnia elegans*.

In *P. grandiflora* (moss rose), even a low concentration of Radifarm® (a 0.25% solution) enhanced germination rate and fresh seedling mass. The effect, however, was stronger in a 0.5% solution: After treating seeds with it, seedling tissues had higher dry matter accumulation than had control seedlings. In *Z. elegans*, seeds treated with the lower Radifarm® concentration showed the highest germination rate and seedling fresh and dry matter. In *H. bracteatum*, the higher biostimulant concentration led to the highest germination rate, whereas both concentrations increased the fresh mass of seedlings.

Parađiković, Vinković, & Radman (2008) conducted the experiment in the optimal germination conditions for the species studied. Thus, the benefits the Radifarm® applied at the germination stage show that biostimulants have much potential for use in ornamental transplant production. Yildirim, Dursun, Güvenç, and Kumlay (2002) reported similar stimulative effects on seed germination in an experiment with parsley, celery, and leek seed soaked with a 1% (v/v) humic acid solution. In the research of Majkowska-Gadomska, Francke, Dobrowolski, and Mikulewicz (2017), ornamental plant species (*Callistephus chinensis* L., *Salvia splendens*, *Zinnia elegans*, and *Tagetes patula* L.) responded differently to tested biostimulants used for seed preconditioning, including Goëmar Goteo (Table 2). Zinnia seeds treated with this biostimulant had the highest germination energy.

4 | CONCLUSIONS AND FUTURE CHALLENGES

A wide array of studies we discussed above have dealt with various biostimulants and their application in a variety of horticultural plant species, in several plant growth phases, from germination to harvest. Their authors analyzed many morphological, physiological, and quality traits of plants. From this plethora of research, one main conclusion follows: In many vegetables, ornamentals, herbs, and medicinal plants, various available commercial biostimulants enhance plant stress tolerance and antioxidative capacity, nutrient acquisition and distribution within the plant, and growth and development of plant vegetative and generative organs. In general, the research presented here confirms the advantages of biostimulant application in horticultural production, especially in stressful growth conditions due to transplantation, reduced fertilization, or other abiotic stress incidences.

Clearly, biostimulants can enhance seed vigor and plant productivity, but to optimize their use, we need to learn a lot about biostimulants themselves—including the chemical composition of commercial products as well as optimal dose and timing—but also about the biochemical processes underlying plant reactions to biostimulant treatment. The available research suggests that particular functional compounds of biostimulants affect seedling and plant growth and development in specific ways, but it is more than likely that their individual compounds interact, resulting in difficult to predict effects without controlled studies. What is more, many experiments suggest that combining several biostimulants can give stronger effects than their individual application. Since biostimulating effects are clearly species-specific and product-specific, what we know about one biostimulant or about one plant species does not directly transfer to another biostimulant or another plant species. To gain both basic and applied knowledge about the effectiveness of biostimulants for a particular plant species, we need to conduct wide-scale research on this species, with various products, treatments, growth stages, etc. This is in concordance with Therond, Duru, Roger-Estrade, and Richard's (2017) four Rs: "… development of operational knowledge about the best management practices to follow to apply the Right product, Right rate, Right time, and Right place." The research performed so far by Croatian and collaborative scientists abroad contribute to better knowledge of the effectiveness and mode of action of plant biostimulants in general. Given the already-proven potential of biostimulants in horticultural production, further research is warranted, but its range seems unlimited.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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