



# Microbial and Plant-Based Biostimulants

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Biostimulants are substances or microorganisms that promote and/or improve plant growth, development, metabolism and/or tolerance to abiotic stress without being fertilizers or pesticides (Zhang and Schmidt 1997; Kauffman et al. 2007; European Biostimulants Industry 2013). The definition of plant biostimulants is flexible, and they are mainly defined by what they are not. Important to mention is that the substances stimulate the plant even in minute amounts (the smallest amounts). In consensus with scientists, regulators and stakeholders, some generally acknowledged main categories of biostimulants have been drawn up (du Jardin 2015; Calvo et al. 2014; Halpern et al. 2015), namely:

- Chitosan and other biopolymers
- Protein hydrolysates and other nitrogenous molecules
- Humic substances
- Seaweed extracts and botanicals
- Beneficial bacteria
- Beneficial fungi
- Inorganic compounds (i.e. Al, Co, Na, Se, Si)

In this chapter, we will only discuss microbial and plant-based biostimulants and their effects on crop quality (for a quick overview, see ■ Tables 12.1 and 12.2).

■ **Table 12.1** List of effects of chitosan and protein hydrolysates on selected crops

Biostimulant	Crop	Effects	Application form	References
Chitosan	Peppermint ( <i>Mentha piperita</i> )	Increased menthol values	Added to cell culture	Chang et al. (1998)
	Oregano ( <i>Origanum vulgare</i> ssp. <i>hirtum</i> )	Increased polyphenol concentration	Foliar spray, 2 weeks before flowering	Yin et al. (2012)
	Sweet basil ( <i>Ocimum basilicum</i> )	Increased polyphenols, terpenoids and antioxidant activity	Seed-soaking and root-dipping before transplantation	Kim et al. (2005)
	Tomato ( <i>Solanum lycopersicum</i> L.)	Increased polyphenolic compounds	Applied to wounds	Liu et al. (2007)
	Apricot ( <i>Prunus armeniaca</i> L.)	Increased total phenols and antioxidant activity	Postharvest fruit coating	Ghasemzad et al. (2010)

Table 12.1 (continued)

Biostimulant		Crop	Effects	Application form	References
		Cherry ( <i>Prunus avium</i> L.)	Enhanced vitamin C synthesis, increased anthocyanin and total phenolic content	Postharvest fruit dipping	Kerch et al. (2011)
		Citrus ( <i>Citrus x nobilis</i> 'W. Murcott')	Improved titratable acidity, ascorbic acidity and water content	Postharvest fruit dipping	Chien et al. (2007)
Protein hydrolysate	Chicken feather	Banana ( <i>Musa paradisiaca</i> L.)	Increased proteins, reduced sugars, amino acids, phenols and flavonoids	Irrigation and foliar	Gurav and Jadhav (2013)
	Plant-derived	Grapevine ( <i>Vitis vinifera</i> L.)	Increased soluble solids, total phenols and anthocyanins	Foliar	Boselli et al. (2015)
	Alfalfa-derived	Pepper ( <i>Capsicum chinensis</i> L.)	Increased several secondary metabolites	Foliar	Ertani et al. (2014)
	Alfalfa-derived	Pecan ( <i>Carya illinoensis</i> )	Increased kernel protein content	Foliar	Ashraf et al. (2013)

Table 12.2 List of effects of humic substances and seaweed extracts on selected crops

Biostimulant	Crop	Effects	Comment	References
Humic substances (humic acid; HA)	Cucumber ( <i>Cucumis sativus</i> L.)	Increased total soluble sugars, reducing sugars	Organic production, greenhouse	Karakurt et al. (2009)
HA	Tomato ( <i>Solanum lycopersicum</i> L.)	Increased soluble solids, ascorbic acid		Yildirim (2007)
HA	Pepper ( <i>Capsicum chinensis</i> L.)	Increased soluble sugars		Karakurt et al. (2009)

(continued)

Table 12.2 (continued)

Biostimulant	Crop	Effects	Comment	References
HA	Grape ( <i>Vitis vinifera</i> )	Improved titratable acidity and soluble solids values	Foliar application at various stages	Ferrara and Brunetti (2010)
Fulvic acid	Lemon tree ( <i>Citrus limon</i> ) on <i>C. macrophilia</i> rootstock	Increased juice pH and vitamin C	Grown on calcareous soil	Sánchez-Sánchez et al. (2007)
Seaweed extracts	Broccoli ( <i>Brassica oleracea</i> var. <i>italica</i> )	Increased antioxidant activity, flavonoids	<i>Ascophyllum nodosum</i> extract	Lola-Luz et al. (2014)
		Increased phenols and isothiocyanate	<i>A. nodosum</i> and <i>Durvillaea potatorum</i> extract	Mattner et al. (2013)
	Cabbage ( <i>Brassica oleracea</i> convar. <i>capitata</i> var. <i>alba</i> )	Increased flavonoids and phenols	<i>A. nodosum</i> extract	Lola-Luz et al. (2013)
	Gram mung bean ( <i>Vigna radiata</i> )	Increased total protein, carbohydrate and lipid content	<i>Sargassum wightii</i> extract	Ashok-Kumar et al. (2012)
	Spinach ( <i>Spinacia oleracea</i> )	Increased antioxidant activity, flavonoid and phenol content and Fe-chelating ability	<i>A. nodosum</i> extract	Fan et al. (2013)
	Olive ( <i>Olea europaea</i> L.)	Increased oil content and linolenic and oleic acid; decreased palmitoleic, stearic and linoleic acid	<i>A. nodosum</i> extract	Chouliaras et al. (2009)

## 12.1 Chitosan

Chitosan is the deacylated derivative of chitin. Chitin is a biopolymer that is a natural compound of fungal cell walls, insect exoskeletons and crustacean shells. Chitosan is easily made by the saponification of chitin and has a better solubility than chitin. Sources are usually food production, i.e. crab or shrimp shell waste, which is demineralized and deproteinized (Rinaudo 2006; Younes and Rinaudo 2015). Chitosan elicits plant defence responses to wounding (Doares et al. 1995) and pathogen infections (Bhaskara Reddy et al. 1999; Bautista-Baños et al. 2003; Yu et al. 2012). The application

of chitosan triggers the oxidative burst response with the production of  $H_2O_2$ , which induces phenylalanine ammonia lyase (PAL) activity (Lee et al. 1999; Zhao et al. 2007). PAL is a plant defence enzyme and is an important enzyme in phenolic compound biosynthesis (Camm and Towers 1973). The increase in phenolic compounds after chitosan treatment has been reported for several horticultural crops including basil (*Ocimum* spp.), grape (*Vitis vinifera* L.) and tomato (*Solanum lycopersicum* L.) (Kim et al. 2005; Meng and Tian 2009; Liu et al. 2007; Badawy and Rabea 2009). Kim et al. (2005) applied chitosan to *Ocimum basilicum* by seed-soaking and root-dipping in a 1% (w/v) solution before transplantation. The treatment leads to increased growth and secondary metabolite content together with a great increase in the levels of human health-promoting rosmarinic acid and eugenol. Eugenol is also an important compound for perfumes. Additionally, polyphenol oxidase (PPO) activity is recorded to be delayed after such treatment (Jiang and Li 2001; De Reuck et al. 2009; Badawy and Rabea 2009). Inhibited PPO activity has several positive effects: a prolonged shelf life attributable to a decreased respiration rate and the inhibited degradation of organic compounds (Qi et al. 2011). This results in a delay of browning and the prevention of weight loss. The most common method to prolong shelf life is fruit dipping/coating. Responses to the application of chitosan depend on the concentration and quality of the product and on the plant species and developmental stage.

## 12.2 Protein Hydrolysates

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Protein hydrolysates (PH) consist of blends of poly—any oligopeptides and amino acids produced from various protein sources by using partial hydrolysis (Schaafsma 2009). Sources can be animal- or plant-derived and are then treated by enzymatic and/or chemical hydrolysis. Therefore, protein hydrolysates are a sustainable solution for agro-industrial waste disposal, e.g. feathers and blood or hay and vegetable by-products (Maini 2006; Kasparkova et al. 2009; du Jardin 2015). PHs stimulate iron (Fe) and nitrogen (N) metabolism, nutrient uptake and water and nutrient use efficiency. This is attributable to the higher soil microbial and enzymatic activity, the improved micronutrient mobility and solubility, the modifications of root architecture and an increase in specific enzymes (Cerdán et al. 2009; Ertani et al. 2009; García-Martínez et al. 2010; Colla et al. 2014; Halpern et al. 2015; Lucini et al. 2015). Plant-derived PHs also modify the phytohormone balance, eliciting auxin- and gibberellin-like effects by specific peptides and phytohormone precursors such as tryptophan (Colla et al. 2014). Many reports have been presented regarding the way that PH can raise the concentrations of human health-promoting phytochemicals such as carotenoids, flavonoids and polyphenols (Parrado et al. 2007; Paradikovic et al. 2011; Ertani et al. 2014). Gurav and Jadhav (2013) reported an accumulation of total phenolics, flavonoids and proteins and an increased antioxidant activity in banana (*Musa* ssp.) after using degraded feather products. In berries of red grapevines (*Vitis vinifera*), increased phenolic (+22%) and anthocyanin (+76%) values have been reported after the treatment of the plants with an enzymatic vegetable extract. This effect is attributable to the phytohormones and nutrients in the extract (Ban et al. 2003; Jeong et al. 2004; Parrado et al. 2007). Ertani et al. (2014) treated pepper (*Capsicum chinensis* L.) with alfalfa (*Medicago sativa*) PH (25 or 50 ml/l). This

led to high concentrations of chlorogenic acid, p-hydroxybenzoic acid and p-coumaric acid in green pepper fruits and to an increase of capsaicin in red pepper fruit. Application of PH from red grapes additionally improved aroma-influencing compounds such as glucose and ascorbate. The results are related to the stimulation of secondary metabolism by an increase of gene expression of phenylalanine ammonia lyase (PAL). PH upregulate the expression and activity of PAL, which promotes flavonoid biosynthesis (Schiavon et al. 2010; Ertani et al. 2011). Ertani et al. (2013) noted a consistent increase in the flavonoid content in hydroponically grown maize under saline conditions after treatment with alfalfa PH in comparison with control plants. Ertani et al. (2013) also described an increase of phenolics induced by salinity (NaCl) but a decrease of phenolics by the PH treatment. Furthermore, Lucini et al. (2015) reported that lettuce (*Lactuca sativa*) grown under saline conditions showed increased terpenes and glucosinolates (among other health-enhancing secondary metabolites) after the application of plant-derived PH. Treatment of lemon balm (*Melissa officinalis* L.) with 2 l/ha of an amino acid mixture via foliar application resulted in enhanced concentrations of several terpenoids (Mehrafarin et al. 2015). In addition, Liu and Lee (2012) have found that PH reduce undesired compounds, e.g. nitrates, in leafy vegetables such as arugula (*Eruca sativa*) and spinach (*Spinacea oleracea*).

A large difference exists between plant- and animal-derived PH with regard to phytotoxicity. Cerdán et al. (2009) have demonstrated no phytotoxic effects of plant-derived PH on tomato (*Lycopersicum* L.), even at the highest concentrations, whereas phytotoxic and growth-depressing effects of animal-derived PH have been reported for fruiting crops after repeated application (Cerdán et al. 2009; Lisiecka et al. 2011). An unbalanced amino acid composition, high concentrations of free amino acids and higher salt concentrations seem to be responsible for the potential detrimental effects of animal-derived PH (Oaks et al. 1977; Moe 2013; Colla et al. 2014). Another negative effect is that root nitrate uptake is repressed because of strong phloem loading with free amino acids (Ruiz et al. 2000). This especially affects plants with a low N supply. The most optimized effects will be achieved by very low dosages. This however depends on the cultivar, environment, phenological stage, time and mode of application (Kauffman et al. 2007; Kunicki et al. 2010; Ertani et al. 2014).

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### 12.3 Humic Substances

Humic substances (HS) are natural compounds of soil organic matter. HS arise from the decomposition of plant, animal and microbial debris and from microbial metabolism. Originally, HS were classified into humins, humic acids and fulvic acids categorized according to their molecular weight and solubility. Schiavon et al. (2010) reported an enhanced expression of PAL that was attributable to HS application and that was accompanied by increased phenol values in leaves of maize (*Zea mays* L.). Stimulation of other compounds linked to the shikimic pathway (alkaloids, tocopherols) has also been noted. A foliar treatment with humic acids (HA) from peat leads to an increase of pyruvic acid in garlic (*Allium sativum*) (Denre et al. 2014). In this case, pyruvic acid is an indicator of pungency and therefore aroma. Moreover, a foliar spray of HA on grape (*Vitis vinifera* L. cv. Italia) improves titratable acidity and soluble solid content and, hence, improves

the taste (Ferrara and Brunetti 2010). As a side effect, berry size is also increased. As HS also carry carboxylic and phenolic functional groups, they are capable of complexing toxic heavy metals and therefore of reducing heavy metal content and mobility in plants (Zeng et al. 2002). Shahid et al. (2012) noted a dose-dependent reduction of  $Pb^{2+}$  uptake by fava bean (*Vicia faba* L.) but only showing high effectiveness when high concentrations of HS were used. HS might also have an indirect effect on fruit quality. A lower incidence of plant disease has been reported, as has the capability of using HS as a carrier for microbial inoculants (Zaller 2006; Singh et al. 2010; Naidu et al. 2013; Canellas et al. 2013). The optimum dosage in general depends on the cultivar and mode of application (foliar spray or soil drench). Additionally, in some cases, the efficacy depends on the source or quality of the HS (Lulakis and Petsas 1995; Azcona et al. 2011).

## 12.4 Seaweed Extracts

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Seaweed extracts are made from marine macroalgae. Several compounds are exclusive to the algal source, and the chemical composition of the extract depends on the extraction method, which in turn affects its biological activity (Kim 2012; Khairy and El-Shafay 2013). In general, the extracts contain polysaccharides, micro- and macronutrients, sterols, betaines, hormones and vitamins and their precursors (Blunden et al. 1985; Berlyn and Russo 1990; Craigie et al. 2008; Khan et al. 2009; Craigie 2011). Of importance to consider, the activity of seaweed extracts is similar to that of phytohormones. At low concentrations, growth increases, but, at high concentrations, growth is inhibited (Provasoli and Carlucci 1974; Khan et al. 2009). Chalcone isomerase (CHI) has been noted to increase after seaweed extract treatment. CHI is a key enzyme for the synthesis of flavonoid precursors. Fan et al. (2011, 2013) have reported enhanced antioxidant activity and flavonoid and phenolic content and improved storage quality in spinach after the application of common brown algae (*Ascophyllum nodosum*) extract. The fatty acid profile of olive oil can also be modified by *A. nodosum* extracts: Chouliaras et al. (2009) noted a significant increase in the health-promoting linolenic and oleic acids and an ample decrease in the rather unhealthy palmitoleic, stearic and linoleic acids. Extract of *A. nodosum*, when sprayed at 10 l/ha/month, has also been found to raise the phenolic and flavonoid content in onion (*Allium cepa*) (Lola-Luz et al. 2014). Lola-Luz et al. (2014) further noted an increase in total phenolic, total flavonoid and total isothiocyanate content in broccoli (*Brassica oleracea* var. *italica*) after such treatment.

An extract of several seaweeds combined (*Sargassum*, *Laminaria*, *A. nodosum*) can be successfully be used as a postharvest treatment that is superior to  $CaCl_2$ , which only maintains the actual quality. Fruit dipping in a 4% seaweed extract results in a significant improvement of sweetness attributable to increases of total soluble solids and sugars and reducing sugars in navel oranges (*Citrus sinensis* (L.) Osbeck) when they are stored at ambient temperature or in cold storage (Omar 2014). Liquid extracts can be applied by irrigation/fertigation or as a foliar spray (Rao 1991; Fornes et al. 2002; Selvaraj et al. 2004; Haider et al. 2012). Foliar application is best performed in the morning when the stomata are open (see ► Chaps. 13 and 20). Responses to seaweed extracts are crop-specific with regard to concentration and frequency of application (Battacharyya et al. 2015).

## 12.5 Botanicals

Botanicals are products derived from plants, algae, fungi or lichens. Sánchez-Gómez et al. (2016) studied effects of extracts from white grape (*Vitis vinifera* cv. Airén) vine-shoot residues as a viticultural biostimulant on grapevine. Results showed an increased varietal aroma typical of the Airén variety (norisoprenoids and terpenes) after foliar application combined with a wetting agent. A positive modulation of the phenolic composition (especially of hydroxycinnamic acid) was also achieved. Moreover, French oak (*Quercus robur*) extract has been noted to influence grape berry volatile organic compounds (VOCs). According to Martínez-Gil et al. (2011), the grapes (*Vitis vinifera* cv. Petit Verdot) store the VOCs primarily as non-volatile precursors, some of which are released after winemaking. The effects were evident only after alcoholic fermentation sampling. Therefore, the results are mainly interesting for young wines, which are bottled immediately after fermentation and clarification. Pardo-García et al. (2013a, b) also found a modulation of the phenolic composition of red wine grapes (*Vitis vinifera* cv. Monastrell). The foliar oak extract treatment resulted in a concentration of polyphenols such as garlic acid, hydroxycinnamoyltartaric acids, acylated anthocyanins, flavonoids and stilbenes in the berries. The application also led to less alcoholic and acid wines, therefore improving taste with a higher colour intensity and deeper shade. Application of lavandin hydrolat (*Lavandula hybrida*) to Petit Verdot grapes exhibited an impact on their wine aroma compounds. Martínez-Gil et al. (2013) showed an unusual increase of camphor in wines after repetitive foliar spraying (5 ×) at weekly intervals, when using 250 ml hydrolat per plant starting at 7 days after the half-veraison. A wetting agent at 0.5 ml/l was added to the hydrolat formulation to ensure sufficient adhesion to the leaves. Additionally, the aroma of the wines was positively modified 6 months after malolactic fermentation. A higher stability of some compounds, i.e. esters, was also discovered.

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