Pacific Northwest Vegetable Association
Kennewick, WA
November 13-14, 2024

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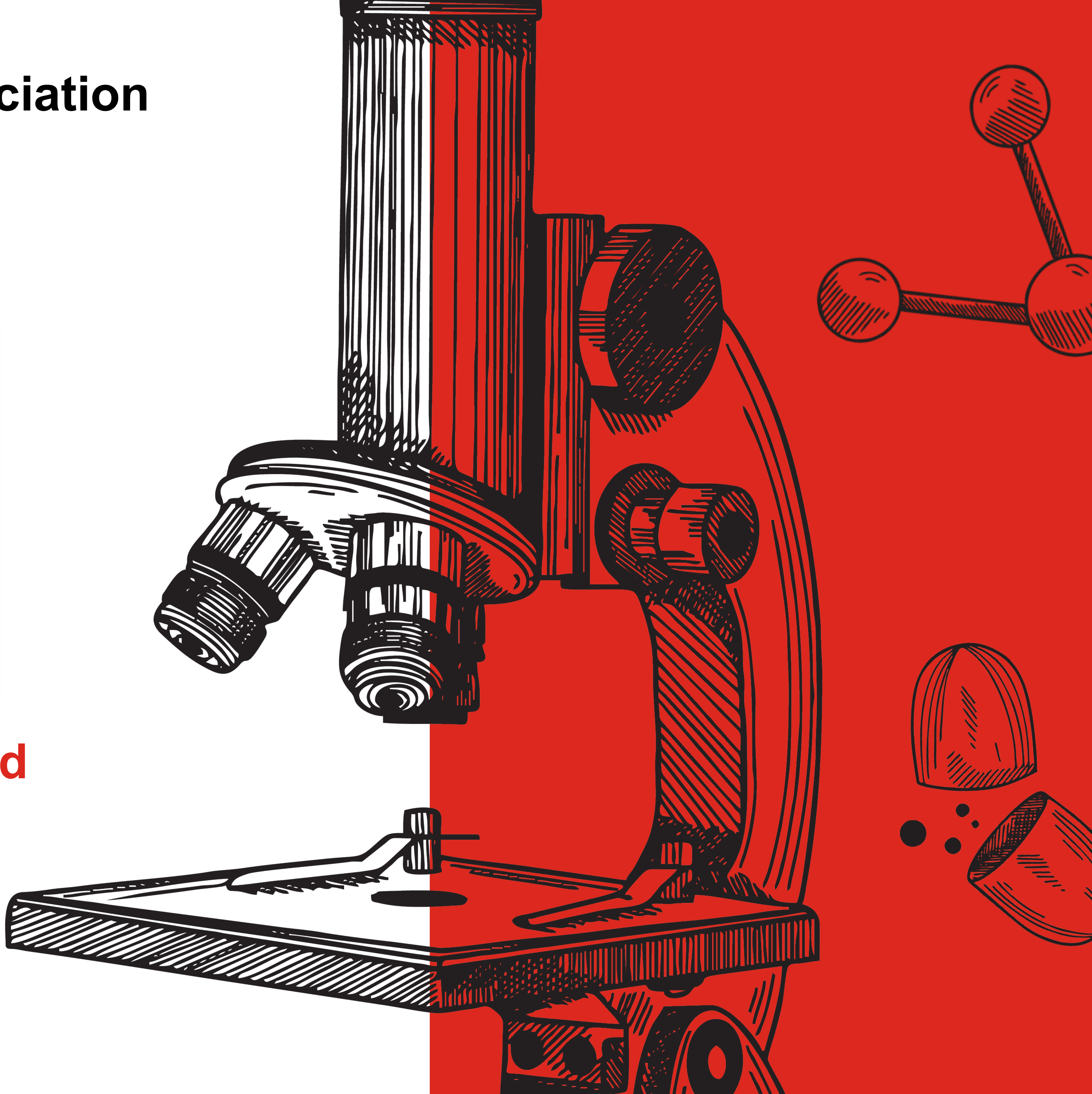
Biostimulants – What They Are and How They Work

Emily Fuerst, R&D Director



Biostimulants Drivers

Demand for sustainable agriculture and high value crops

Potential for biostimulants to help bridge the gap(s)

History of improving plant nutrition, quality, yield, and abiotic tolerance in different agricultural crops

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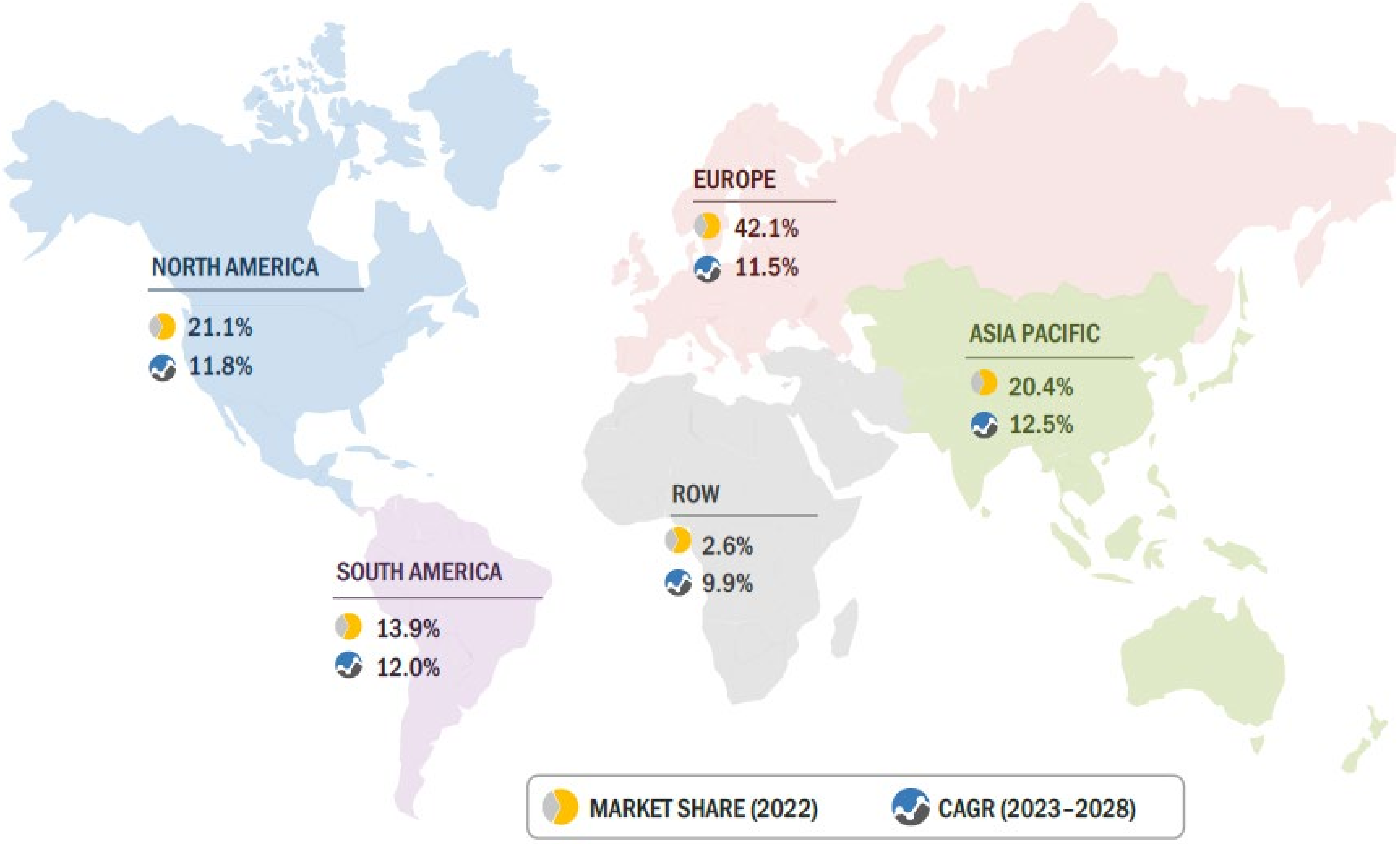
Biostimulants Market



Biostimulants Market Global Forecast to 2028. MarketsandMarkets, November 2023

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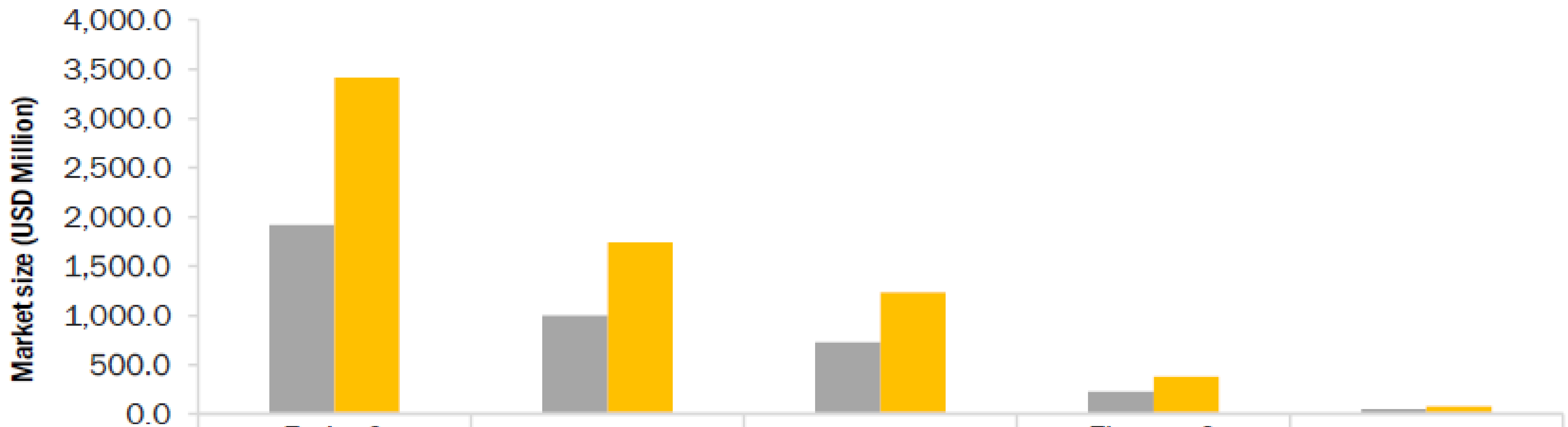
Biostimulants Market



	Fruits & Vegetables	Cereals & Grains	Oilseeds & Pulses	Flowers & Ornamentals	Others Crop types
2023	1,919.1	998.7	727.3	224.1	48.8
2028	3,413.3	1,739.9	1,234.8	379.5	79.7

Biostimulants Market Global Forecast to 2028. MarketsandMarkets, November 2023

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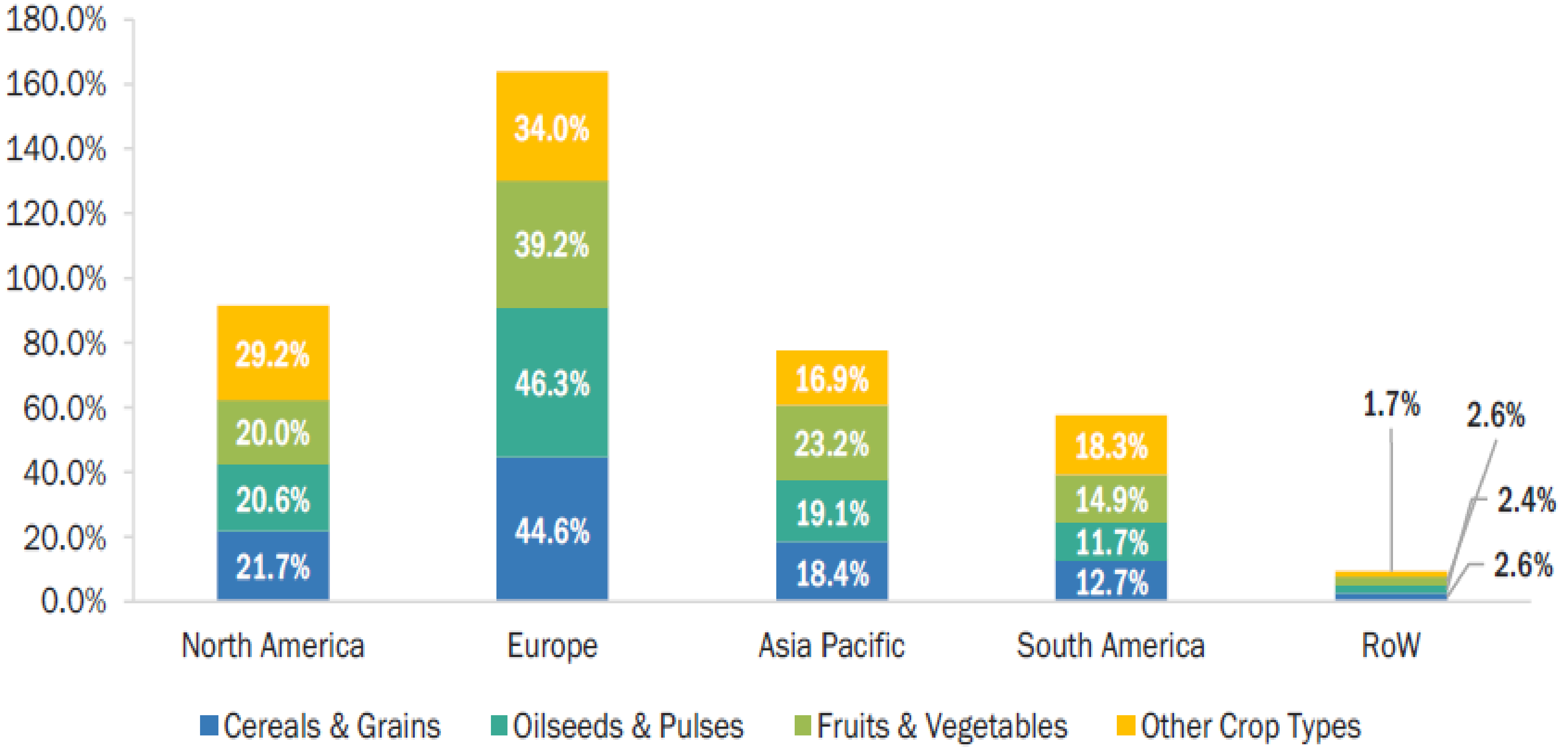
Biostimulants vs PGR

- Europe led the way with early adoption and guidance…
- US confusion regarding regulations for biostimulants
  - **Pesticide/PGR or not?**
  - This has slowed the introduction to the US market
- US guidance is now available but still can change.

2023 Biostimulants by Crop Type and Region



Biostimulants Market Global Forecast to 2028. MarketsandMarkets, November 2023

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What is a Plant Growth Regulator?

- FIFRA §2(u) defines **plant regulators as pesticides**
  - Defines "pesticide" to include not only those substances that are intended to "prevent, destroy, repel, or mitigate" pests, but also a wide range of other products such as defoliants, desiccants, nitrogen stabilizers, and plant growth regulators (PGRs).
- FIFRA §2(v) defines plant regulators and substances excluded from definition of a plant regulator
  - "…any substance or mixture of substances intended, through physiological action, for accelerating or retarding the rate of growth or rate of maturation, or for otherwise altering the behavior of plants or the produce thereof…"
  - **It does not include:**
    - plant nutrients/trace elements*
    - nutritional chemicals (not defined in FIFRA or CFR)
    - plant inoculants*
    - soil amendments*
    - vitamin-hormone horticultural products**

**40 CFR 152.6(g)(1), (2), & (3) 5*
***40 CFR 152.6(f)(1) & (2)*

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What is a Biostimulant?

"…a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield."

USDA, December 20, 2019

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How are each regulated?

Biostimulants "Non-pesticidal" Claims*	Plant Growth Regulator "Pesticidal" Claims
• Avoids/corrects/prevents nutrition-based/nutrient deficiency-based plant disorders (e.g., including, but not limited to: blossom end rot, chlorosis, necrosis, discoloration, stunting, etc.) • Improves soil/seed nutrient conditions for root growth • Improve/increase/support biodegradation of organic matter • Increases/improves/optimizes soil conditions for increased plant vigor • Increases/improves/optimizes conditions for tolerance of/resistance to abiotic stress • Improves overall plant nutrition • Supports nutrient uptake	• Enhances/promotes/stimulates fruit growth and development • Inhibits/promotes sprouting • Induce/promote/retard/suppress seed germination • Enhances/promotes crop/fruit/produce color/development/quality/shape

*certain claims for **increased or decreased plant growth, yield, or germination** can be made without triggering pesticide regulation if they are *consequent to the intended use as an exception to the definition of a PGR* (for example, as a **plant nutrient, inoculant**, etc.)

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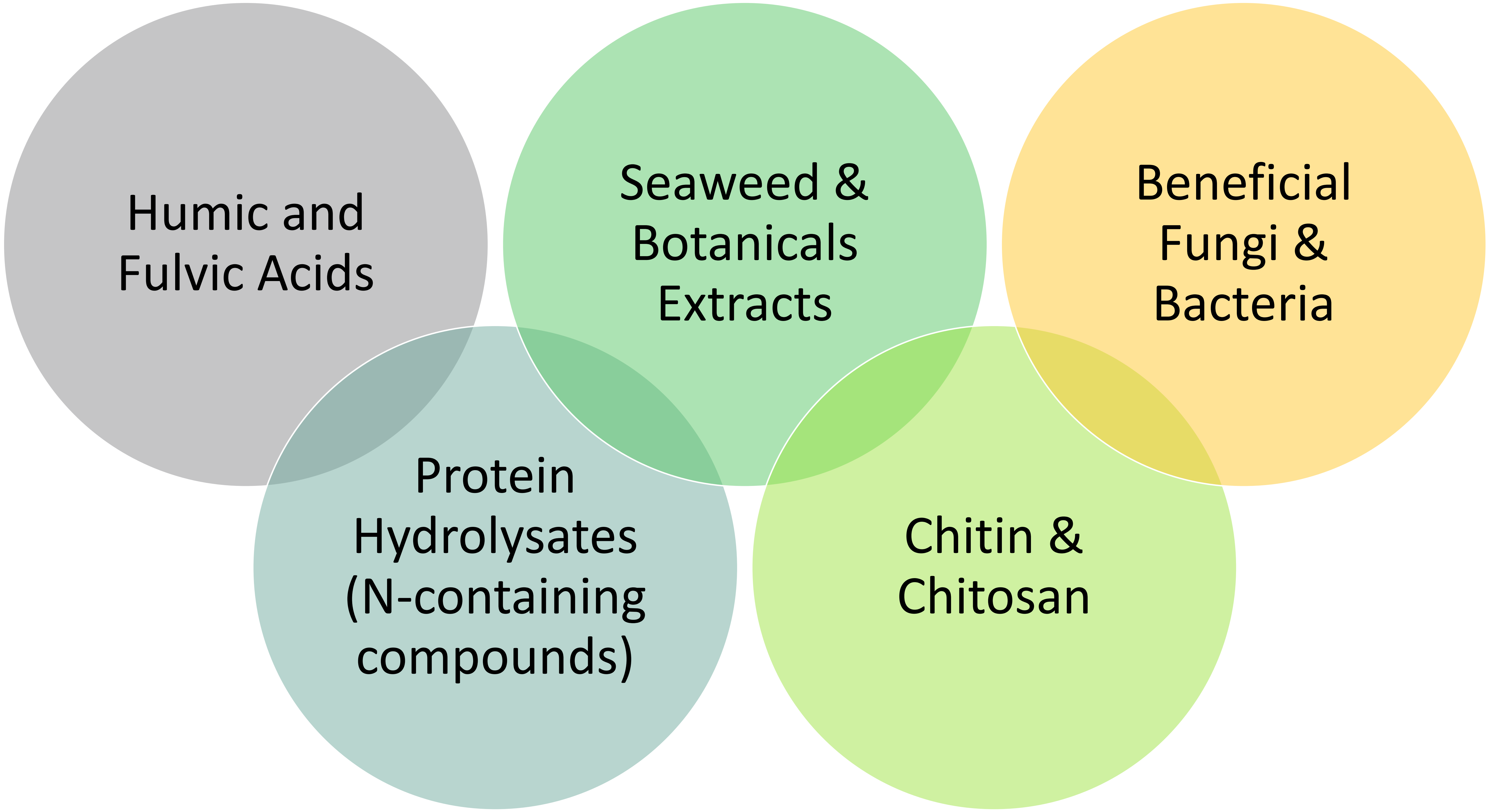
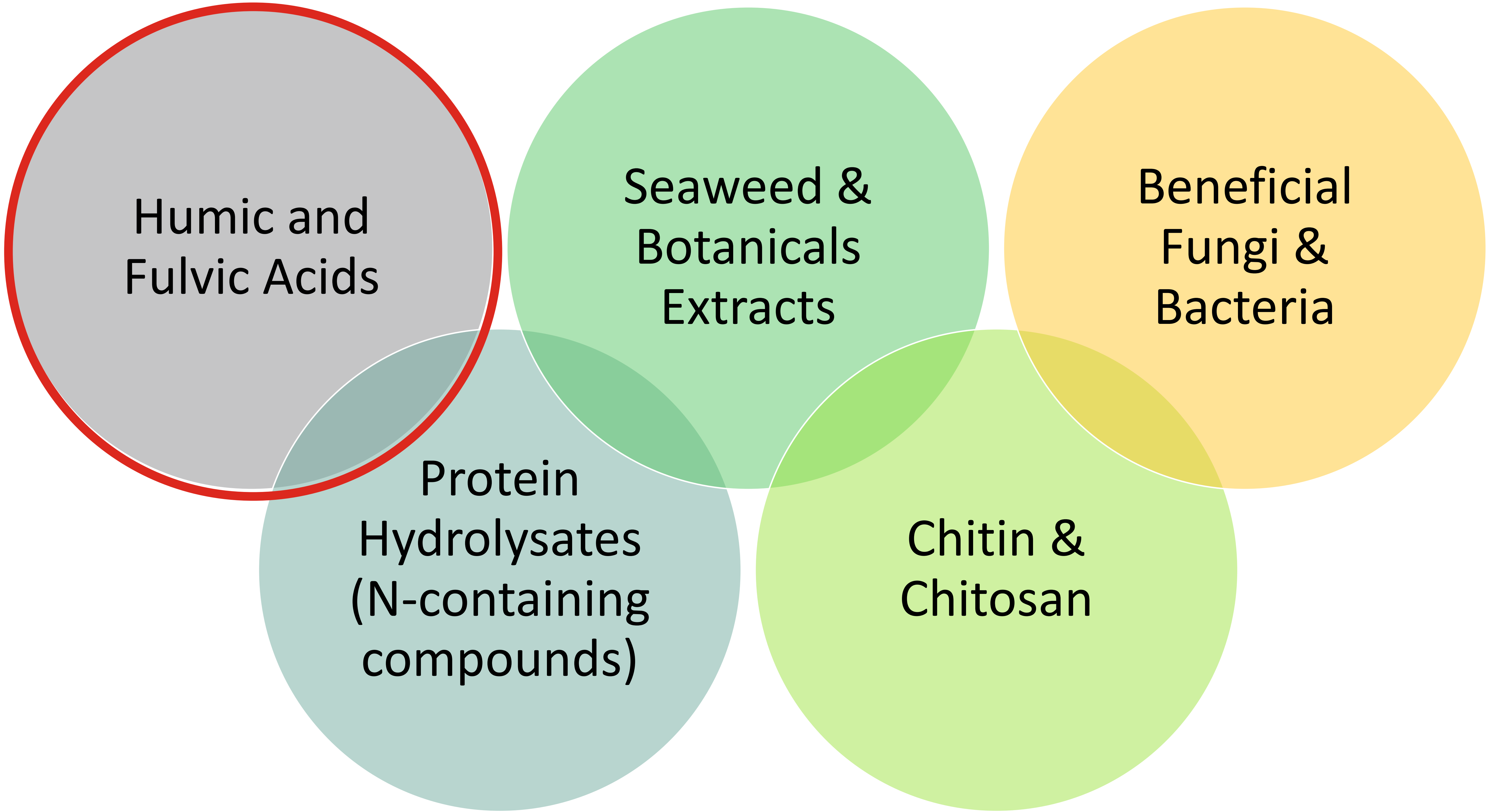
Biostimulant Categories



Biostimulant Categories



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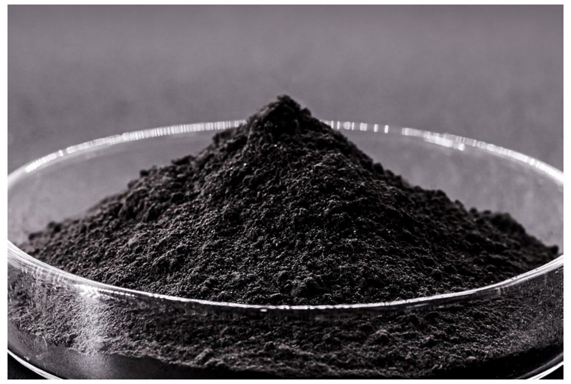
Humic & Fulvic Acids

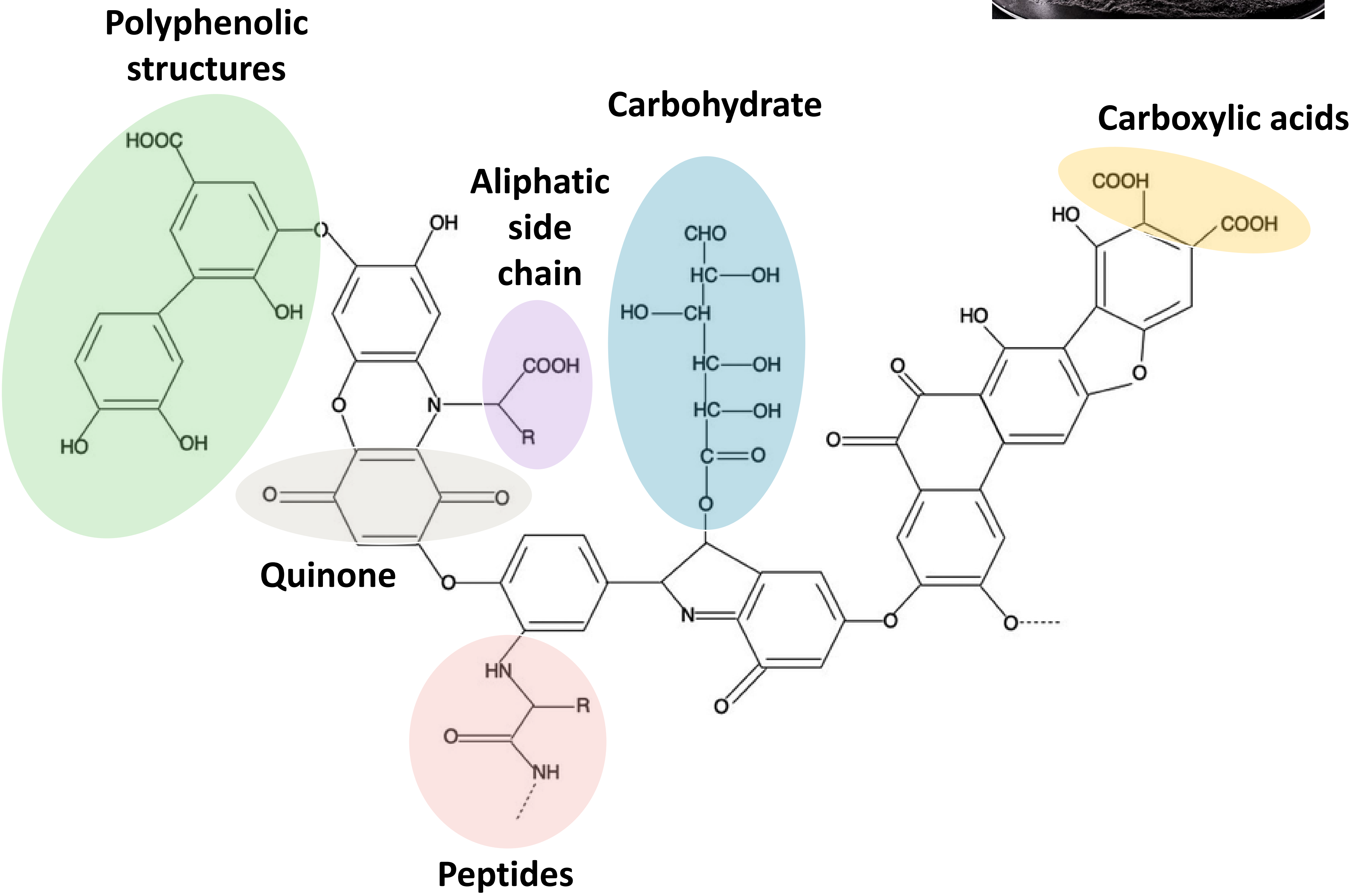


Dead animals and plants that undergo humification

- transformed over time from multiple cycles of consumption and processing resulting in humus = humic acids, fulvic acids, humin
- Sourced from coal, lignite, soils and organic materials

Humic Acid

- Complex chemical structure of multiple carboxylic acid side chains, heterocyclic chemicals, and multi-aromatic components
- Molecular weight = 1,000 – 30,000 Da
- Soil conditioning: redox buffering, pH buffering, strong ion binding, hydrophilic/hydrophobic
- Soluble only in alkaline solutions



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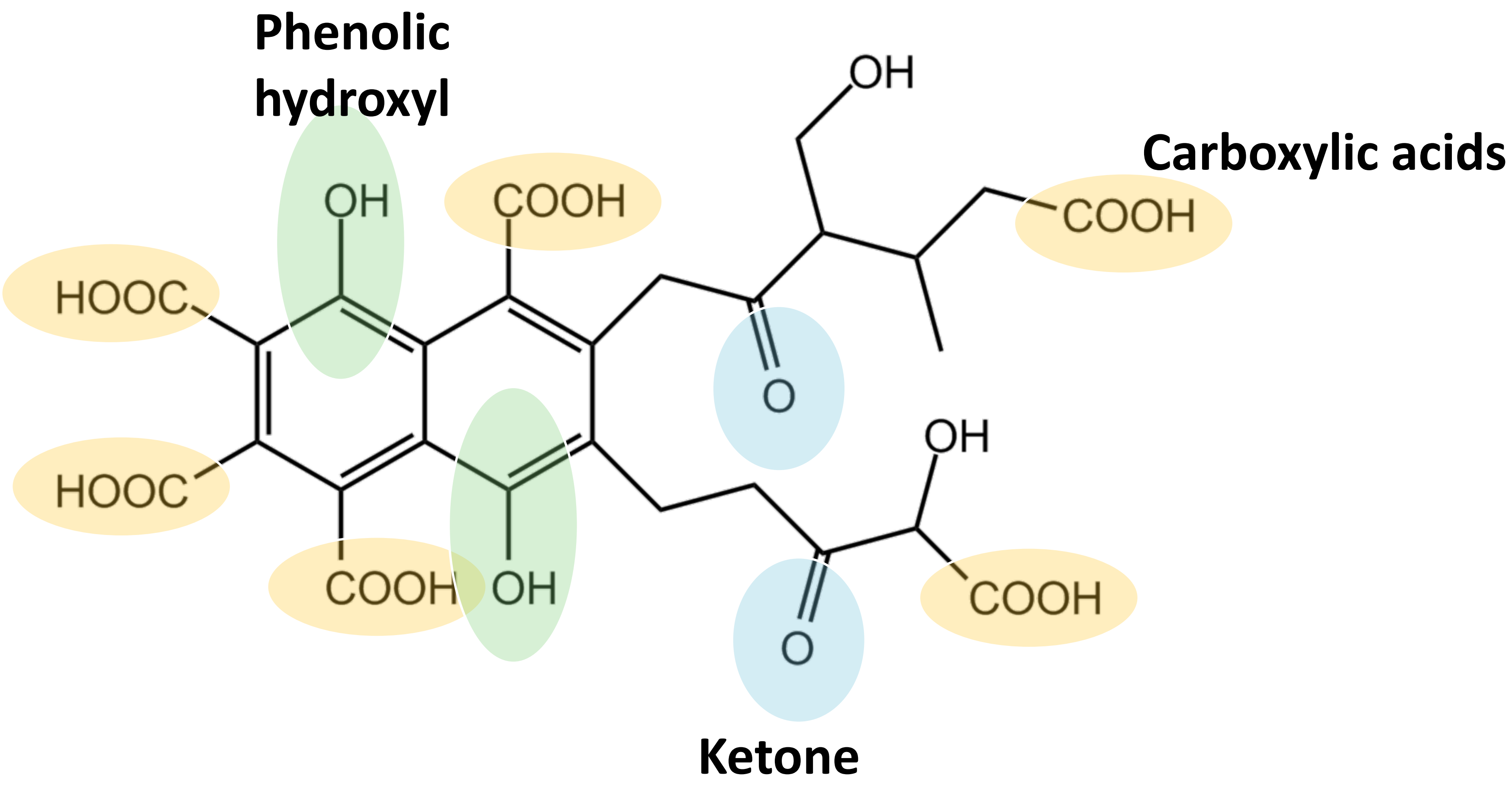

Fulvic Acid

- Aromatic organic polymers, many carboxyl groups, phenolic hydroxyl groups, and oxygen-rich functional groups
- Molecular weight = 500 – 1,000 Da
- Soil conditioning: pH buffering, mineral/ion binding, hydrophilic
- Soluble in water at all pH values

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Humic & Fulvic Acids

- Improves soil properties
  - **Water holding capacity**
  - **Cation exchange capacity**
  - **Soil structure**
  - **Microbial activity**
- Nutrient uptake
  - **Binding of macro/micro-nutrients such as Ca, P, K, Zn**
  - Root growth and stimulation of plasma membrane H^+-ATPases (increase electrochemical potential used for import of nitrate and other nutrients)[2,3]
- Crop yield enhancement
  - Results of improved growth parameters and nutrient absorption
- Stress Resistance
  - Salinity and heat tolerance by improving root development and plant enzyme activity

1) Bhatt, Pooja & Singh, V. (2022). Effect of humic acid on soil properties and crop production– A review. The Indian Journal of Agricultural Sciences. 92. 10.56093/ijas.v92i12.124948.
2) Calvo P, Nelson L, Kloepper JW(2014) Agricultural uses of plant biostimulants. Plant Soil 383:3–41. https://doi.org/10.1007/s11104-014-2131-8
3) Mora V, Bacaicoa E, Zamarreño A-M et al (2010) Action of humic acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients. J Plant Physiol 167:633–642

Humic & Fulvic Acids

Impact on plant growth and soil properties

Crop	HA dose	Result	Reference
Soil aggregates	100–200 kg/ha	Increased the stable soil aggregates range from 40–120% than control.	Piccolo *et al*. 1996
Soil moisture content	0.05 g/kg	Significantly increased the field capacity (25.9%), permanent wilting point (21.7%), and available water content (29.9%) in Acireale soil than control.	Piccolo *et al*. 1996
Chickpea (*Cicer arietinum* L.)	9 kg/ha	Increased the number of branches and pods per plant and seed yield.	Kahraman 2017
Sugarcane (*Saccharum officinarum* L.)	20 kg/ha 30 kg/ha	Catalase and alkaline phosphate activity Dehydrogenase activity	Sellamuthu and Govinda Swamy 2003
Mustard (*Brassica compestris* L.)	6.35 kg/acre	Increased growth and yield	Rajpar *et al*. 2011
Fodder maize (*Zea mays* L.)	25 kg/ha	Improved growth and quality of fodder maize	Daur and Bakhashwain 2013
Wheat (*Triticum aestivum* L.)	150 mg/kg	Increased shoot length 18%, root length 29%, and yield 19–55% as compared to control.	Arjumend *et al*. 2015
Tobacco (*Nicotiana tabacum* L.)	14.8 kg/ha	Decreased the concentration of Pb, Cd, Zn and Cu by 39, 37%, 29%, and 18%, respectively, as compared to control.	Rong *et al*. 2020

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Humic & Fulvic Acids

Impact on nutrient uptake & plant growth

Crop	Type of Humic Substance	Reference	Study Conditions	Reported Effects on Growth and Nutrient Uptake
Cucumber	Humic acid	El-Nemr et al. 2012	Field tests in two years with foliar sprays	Increased plant growth and yield; enhanced uptake of N, P, K, Ca, and Mg
Cucumber	Humic acid	Karakurt et al. 2009	Yield and fruit- quality study in ground in organic production greenhouse conducted in two years	Increased total fruit yield, total soluble sugars, reducing sugars, and chlorophyll b
Cucumber	Humic acid	Mora et al. 2010	Hydroponic culture in growth chamber	Increased shoot growth; increased NO_3 in shoots and decreased NO_3 in roots
Potato (Solanum tuberosum)	Humic acid	Selim et al. 2012	Field study with different water regimes; application through fertigation system	Enhanced tuber yield; increased percent protein and ascorbic acid content in tubers; increased SPAD readings (chlorophyll indicator) in leaves
Tomato	Humic acids	Adani et al. 1998	Hydroponic culture	Increased growth of roots and shoots; enhanced uptake of N, P, Fe, and Cu
Tomato	Humic acid	Yildrim 2007	In-ground greenhouse test for yield conducted during two growing seasons	Increased early and total yield in both years; increased total soluble solids and ascorbic acid content in fruit
Tomato	Fulvic acid and humic acid	Lulakis and Petsas 1995	Growth chamber tests with seedlings in Petri plates	Enhanced root and shoot growth at 14 days after seeding
	Fulvic acid and humic acid	Chen et al. 2004		

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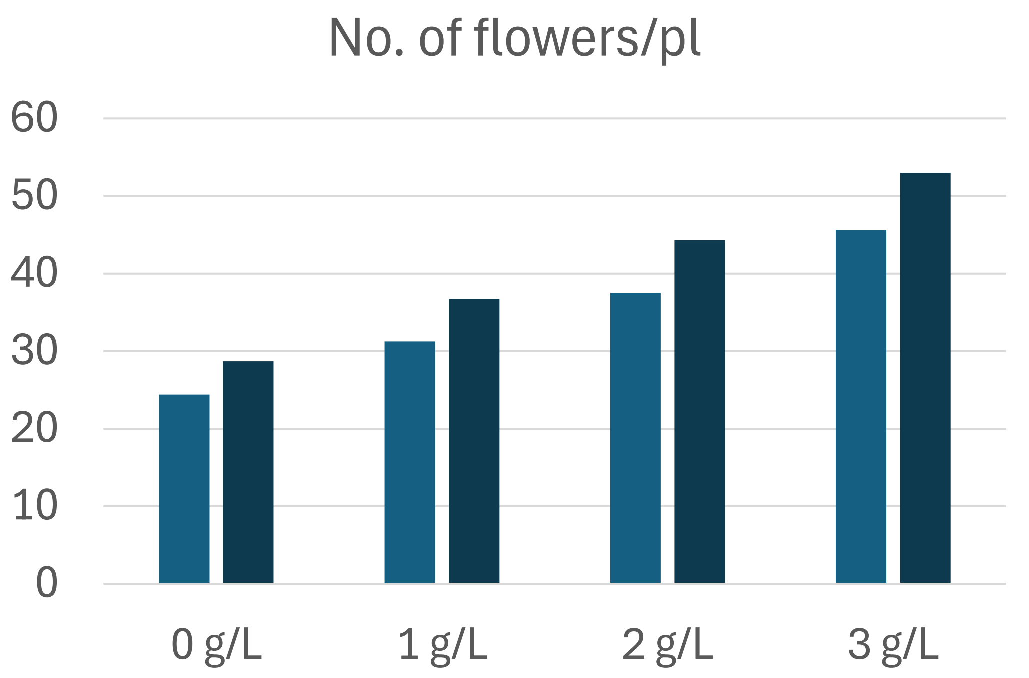
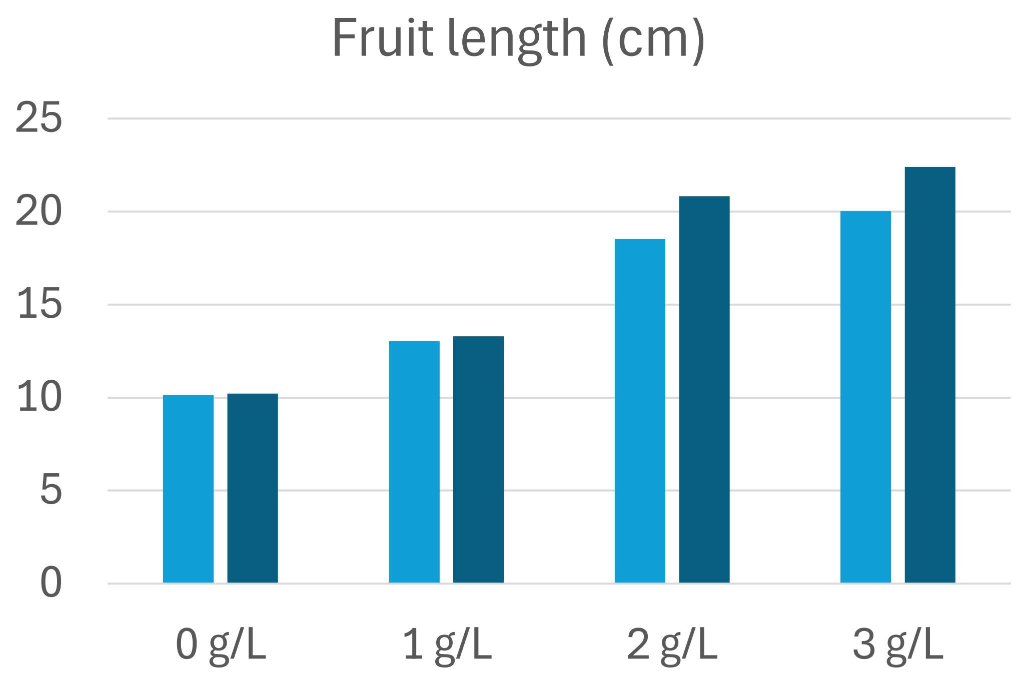
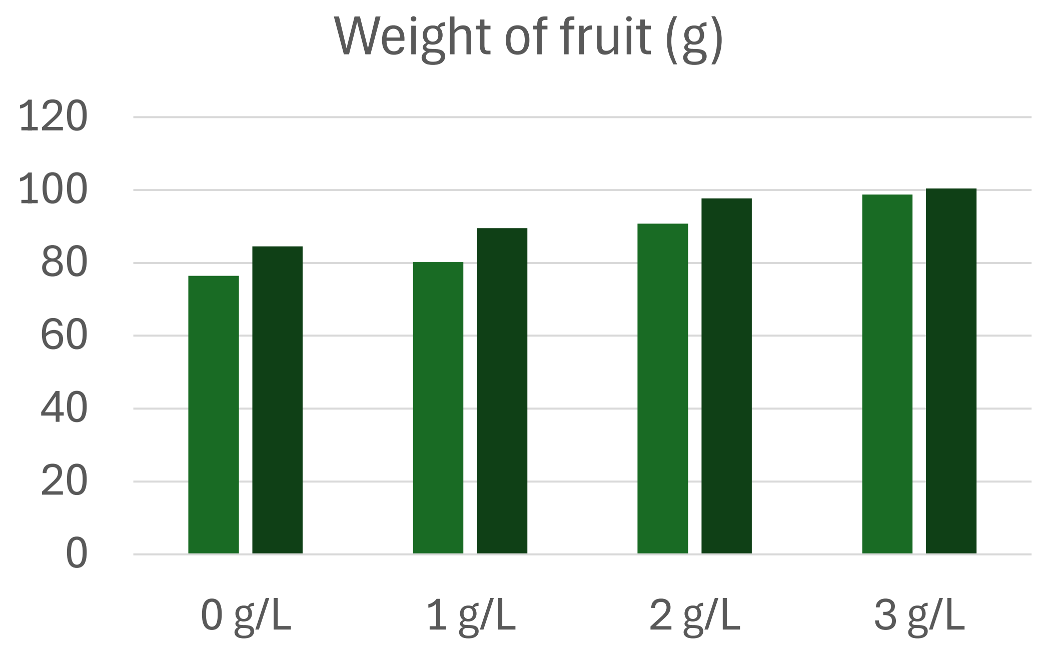
Humic Acids – on Cucumber

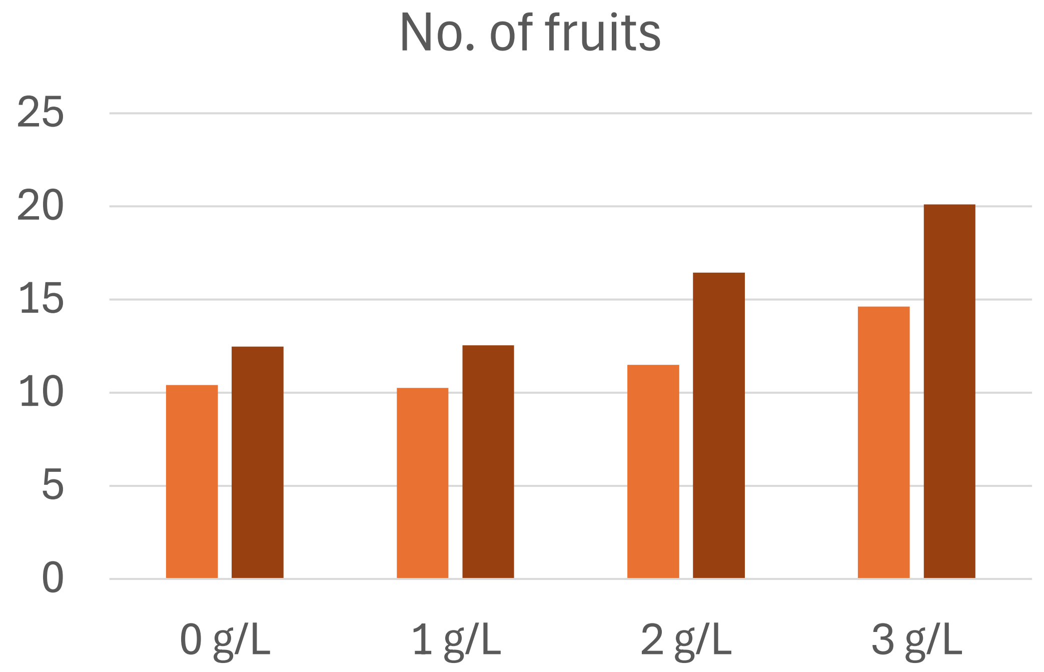
El-Nemr et al (2012)

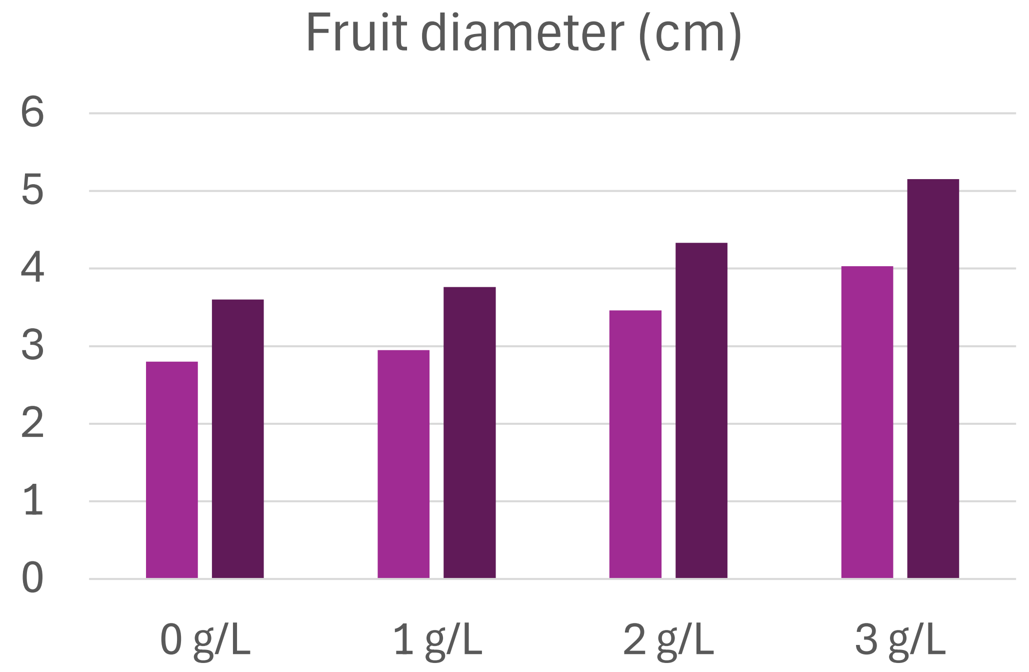
Random complete block design. N=3 per treatment.
Foliar applied at 3 rates (1, 2 or 3 g/L) and a negative control (0 g/L).
Applied 3 times at 15-day interval, starting 3 weeks after planting

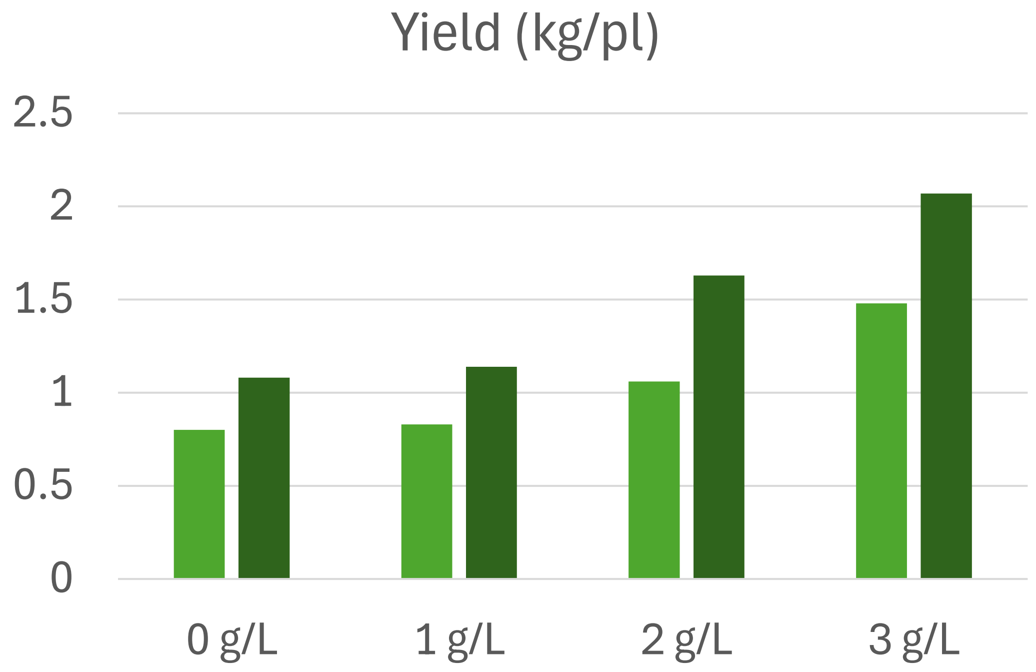












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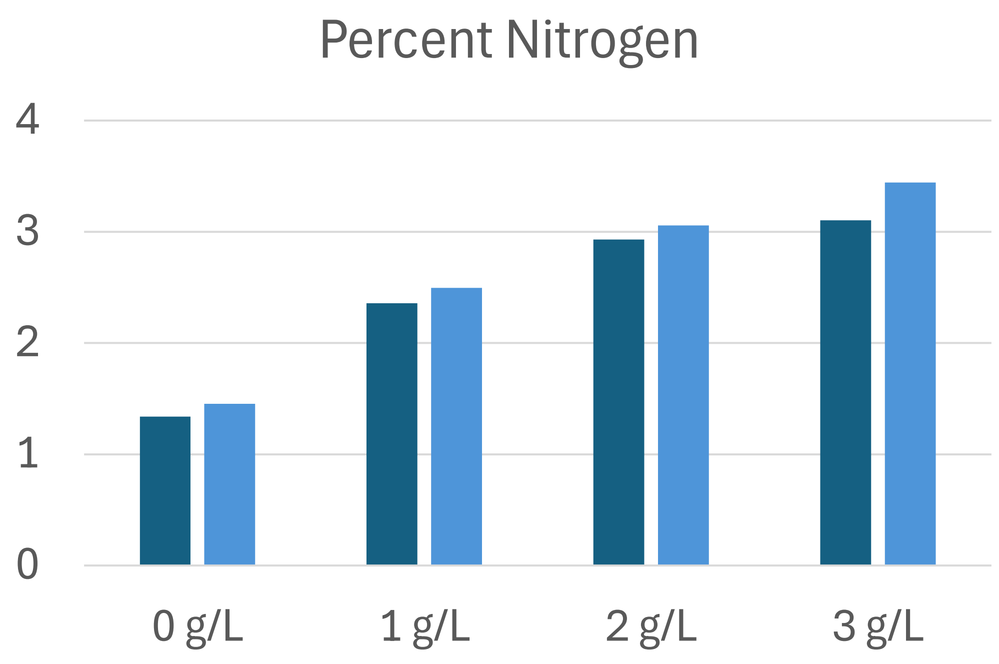

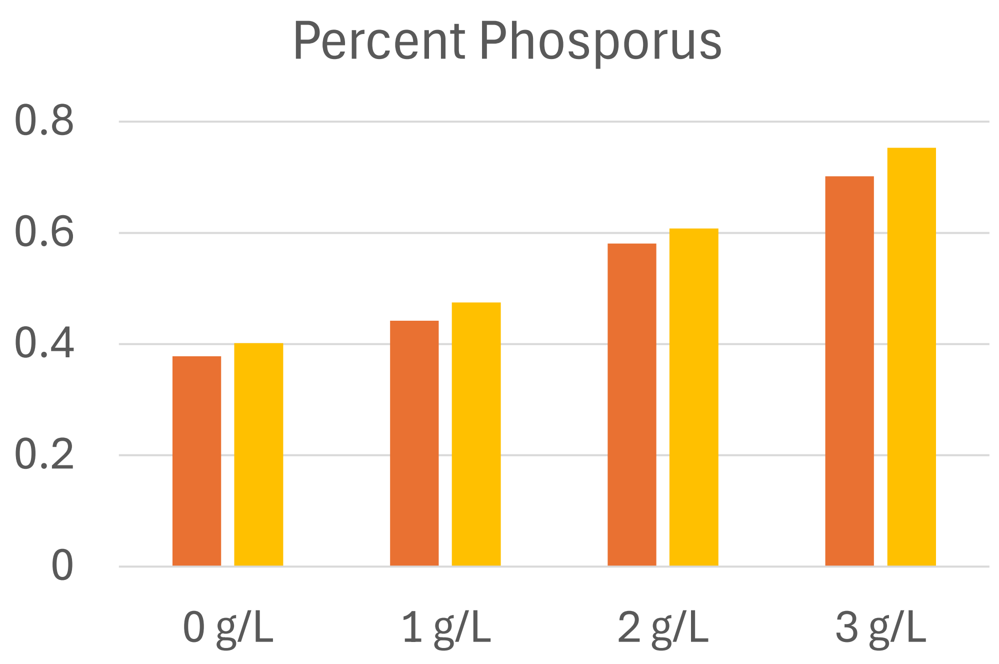

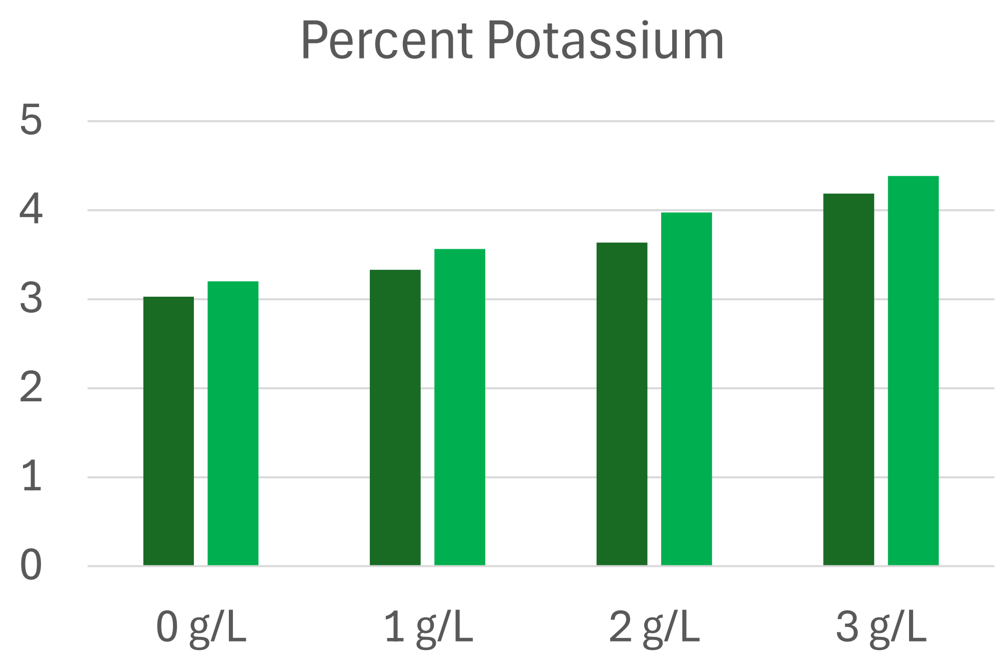

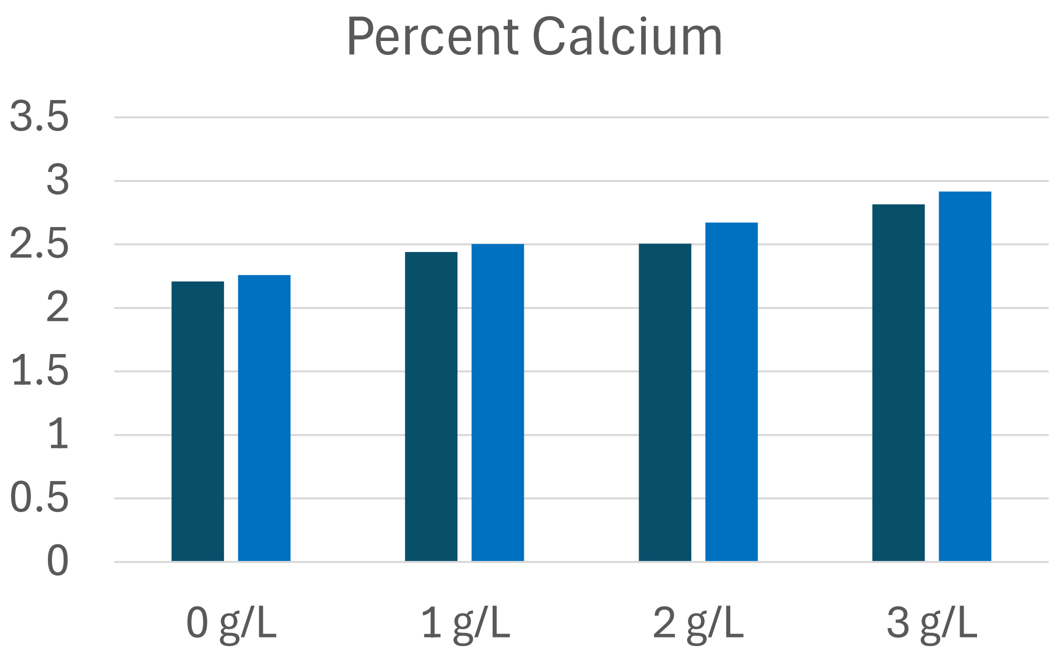

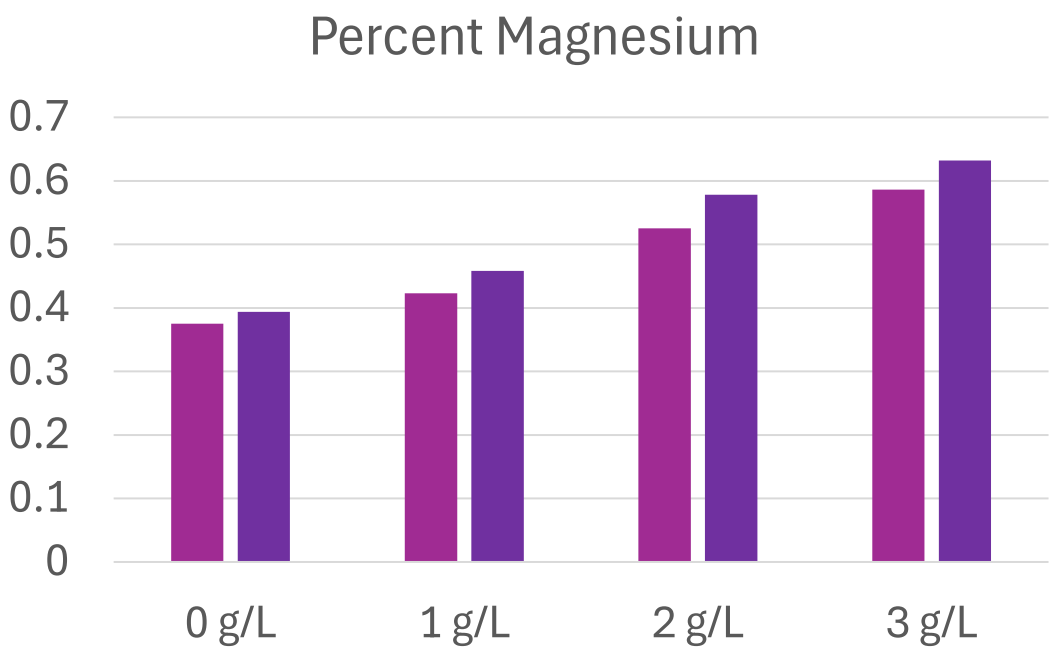

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Humic Acids – on Potato

Selim et al. (2012)

Treatments	Leaf area (m^2)	Dry foliage weight (g plant^{-1})	Relative water content (%)	Tuber production (Mg ha^{-1})
Mean values as affected by water stress				
Water unstressed plants	0.338	37.79	77.99	40.33
Water stressed plants	0.318	36.84	69.77	33.63
F test	--	--	--	*
Mean values as affected by humic acid fertigation levels				
Without	0.323	34.32c	71.94c	34.32c
60 kg ha^{-1}	0.33	37.66b	73.84b	37.66b
120 kg ha^{-1}	0.331	39.98a	75.87a	39.98a
Water unstressed plants				
Without	0.338	34.65c	76.98c	36.56c
60 kg ha^{-1}	0.339	37.86b	77.00b	40.87b
120 kg ha^{-1}	0.338	40.87a	79.98a	43.56a
Water stressed plants				
Without	0.308	33.98d	66.89d	32.87d
60 kg ha^{-1}	0.321	37.45cd	70.68cd	33.78d
120 kg ha^{-1}	0.324	39.08c	71.75cd	34.23cd

Treatments	Leaf Chlorophyll (SPAD)	Ascorbic acid (mg 100g FW-1)	NO^- content (mg Kg^{-1})	TSS %	Starch %	Protein %
Mean values as affected by water stress						
Water unstressed plants	43.18	97.46	83.5	5.2	13.71	13.15
Water stressed plants	40.62	76.07	71.14	5.34	13.7	11.71
F test	*	*	*	--	--	--
Mean values as affected by humic acid fertigation levels						
Without	40.16b	77.98c	69.28	5.07	13.38	11.95b
60 kg ha^{-1}	41.79a	82.55b	77.62	5.32	13.75	12.66a
120 kg ha^{-1}	43.76a	99.77a	85.06	5.44	13.99	12.68a
Water unstressed plants						
Without	40.45c	87.07c	76.08	5.03b	13.65b	12.54b
60 kg ha^{-1}	43.34ab	94.65b	86.65	5.20ab	13.63b	13.45a
120 kg ha^{-1}	45.76a	110.67a	87.76	5.37ab	13.86a	13.46a
Water stressed plants						
Without	39.87d	68.89d	62.47	5.10ab	13.10c	11.35d
60 kg ha^{-1}	40.23c	70.45e	68.58	5.43ab	13.87a	11.87c
120 kg ha^{-1}	41.76b	88.86b	82.36	5.50a	14.12a	11.90c

Selim EM, Shaymaa IS, Asaad FF, El-Neklawy AS (2012) Interactive effects of humic acid and water stress on chlorophyll and mineral nutrient contents of potato plants. J Appl Sci Res 8:531–537

Humic Acids – on Tomato

Yildrim (2007)

Treatment Design:

- Rates: 0, 10, or 20 ml/L
- Foliar or drench applied
- Applied 4x starting 3 wk after planting in 10-day intervals

Results:

- No effect on pH and titratable acidity (TA)
- Increased total soluble solids (TSS) with both foliar and soil applications
- Higher rate with foliar application gave highest ascorbic acid (AA) and yield

	pH		AA (mg/100 ml)		TSS (%)		TA (%)		Leaf dry matter (%)		Stem dry matter (%)	
Treatment	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Control	4.69	4.78	47.02 d*	33.16 c*	5.6 b*	5.5 b*	1.17	1.18	13.12 c*	11.11 b*	19.62 c*	19.06
Foliar 10 ml/l	4.57	4.72	59.70 b	46.23 b	6.4 a	6.2 a	1.21	1.18	14.30 ab	12.61 a	21.51 ab	19.23
Foliar 20 ml/l	4.59	4.62	65.57 a	53.45 a	6.6 a	6.5 a	1.22	1.21	14.96 a	12.67 a	22.21 a	19.73
Soil 10 ml/l	4.54	4.60	52.69 c	42.06 b	5.9 b	6.5 a	1.24	1.11	13.42 bc	11.79 ab	20.69 abc	19.23
Soil 20 ml/l	4.49	4.58	54.25 c	44.82 b	6.5 a	6.5 a	1.07	1.19	13.42 bc	11.87 ab	20.13 bc	19.22
LSD (0.05)	n.s	n.s	3.11	4.60	0.32	0.36	n.s	n.s	1.01	1.26	1.63	n.s.

	Fruit diameter (cm)		Fruit height (cm)		Mean fruit weight (g)		Fruit number (per plant)		Early yield (g/plant)	
Treatment	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Control	6.25 c*	7.18 b*	5.13 c*	5.45 b*	88.49 b*	110.57 d*	48 c*	54 d*	935 c*	1676 b*
Foliar 10 ml/l	6.69 ab	7.43 a	5.47 ab	5.69 a	94.44 a	114.63 c	60 a	65 ab	1253 ab	1869 ab
Foliar 20 ml/l	6.83 a	7.45 a	5.63 a	5.76 a	96.46 a	119.73 a	63 a	70 a	1379 a	1949 a
Soil 10 ml/l	6.50 b	7.35 ab	5.20 c	5.76 a	87.63 b	110.57 d	52 b	58 cd	1175 b	1695 b
Soil 20 ml/l	6.66 ab	7.25 ab	5.36 bc	5.70 a	94.48 a	117.60 b	55 b	62 bc	1137 b	1801 ab
LSD (0.05)	0.21	0.24	0.23	0.31	3.47	1.99	3.55	4.66	179	196

Yildrim E (2007) Foliar and soil fertilization of humic acid affect productivity and quality of tomato. Acta Agric Scand 57: 182–186, Sect. B

Biostimulant Categories

Humic and Fulvic Acids

Seaweed & Botanicals Extracts

Beneficial Fungi & Bacteria

Protein Hydrolysates (N-containing compounds)

Chitin & Chitosan

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Protein Hydrolysates (Nitrogen sources)

Amino acids and protein hydrolysates…

- Obtained by:
  - Chemical and enzymatic protein hydrolysis from by-products like plant and animal wastes
  - Chemical synthesis or fermentation for single and mixed compounds.
- Impacts on:
  - Modulation of nitrogen uptake in roots and assimilation via regulation of enzymes
  - **Acts on the signaling pathways of nitrogen acquisition in roots**
  - **Regulates enzymes of the TCA cycle - impact on C and N metabolism**
  - **Select forms can chelate or contain high levels of proline** – heavy metal protection, micronutrient mobility/acquisition, pollen fertility, water balance regulation
  - Environmental stress reduction via antioxidant activity – ie. glycine betaine, proline
  - Soil microbiome increase in mass and activity

Amino acids and peptides can penetrate and diffuse into plant tissues (leaves) through membrane pores and is energy-dependent

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Protein Hydrolysates

Different sources…

Animal Origin	Plant Origin
• Leather by products • Blood meal • Fish by-products • Chicken feathers • Casein	• Legume seeds • Alfalfa hay • Vegetable by-products • Yeast

Protein Hydrolysates

Processing makes a difference…

Chemical Hydrolysis	Enzymatic Proteolysis
• Via acid or alkaline treatment at high temperature (>121 °C) and pressure (>220 kPa) • High content of free amino acids • Destruction of some amino acids (Lys, Trp, Ser, Thr, Cys) • Conversion from L-form to D-form (limiting their metabolism, potential toxic effects)	• Via controlled conditions • Selective peptide cleavage via proteases – high variability in resulting biostimulant properties • Maximized peptide/oligopeptide proportion and yields • Have good solubility, diffusion, and absorption

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Protein Hydrolysates - Lettuce

Lucini et al. (2015)

Design

- Biostimulant: enzymatically digested vegetal protein hydrolysate
- RCBD of 4 replicates of 10 plants/T
- Salinity: 1 mM or 25 mM NaCl
- Treatments: applied weekly
  - Control
  - Root (100 ml of 2.5 ml/L)
  - Root (100 ml of 2.5 ml/L) + foliar (2.5 ml/L)

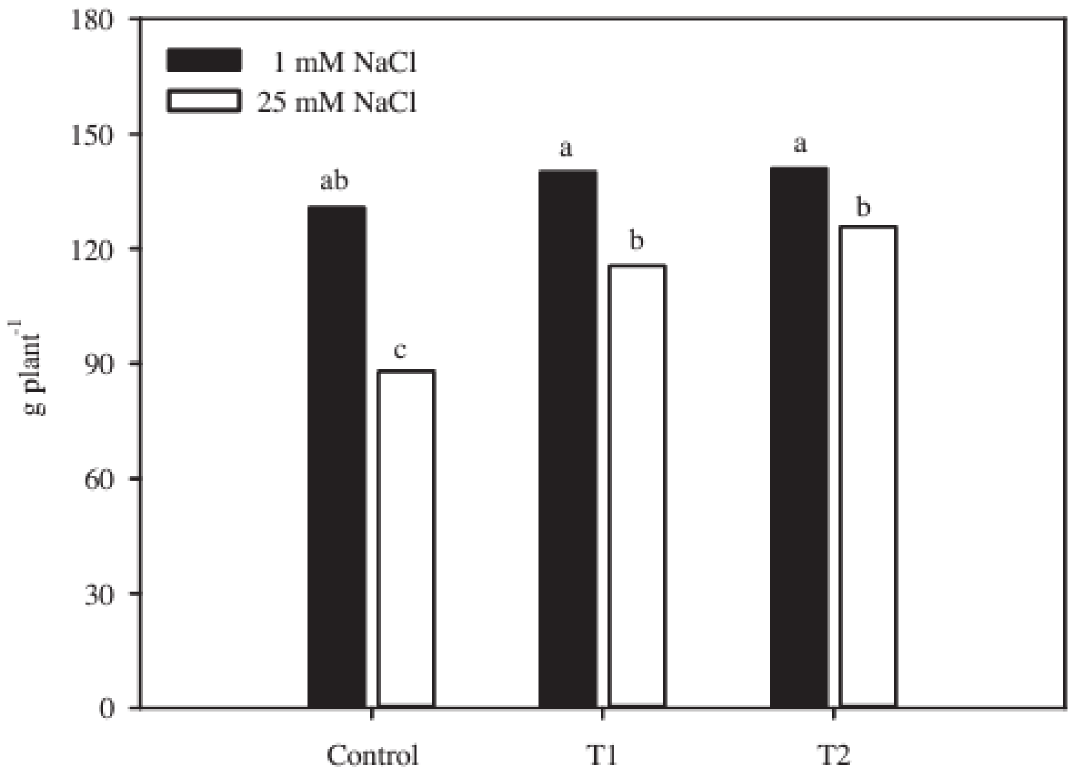

Fig. 1. Effect of biostimulant application to roots (T1) and to roots and leaves (T2) on shoot fresh weight of lettuce plants grown under two saline levels. Different letters indicate significant differences according to Duncan's test ($P = 0.05$). Values are the means of three replicate samples.

Treatments	Shoot dry weight ($g\ plant^{-1}$)	Root dry weight ($g\ plant^{-1}$)	Root to shoot ratio	Total root length ($m\ plant^{-1}$)	Root diameter (mm)	Total root surface ($m^2\ plant^{-1}$)
Salinity (mM NaCl)						
1	7.16 a	1.59 a	0.22 a	322.5 a	0.29 a	0.30 a
25	6.65 b	1.50 b	0.23 a	317.6 a	0.28 a	0.28 b
Biostimulant						
Control	6.22 b	1.14 b	0.18 b	243.6 b	0.28 b	0.21 b
Root application	7.15 a	1.59 ab	0.22 ab	308.7 ab	0.30 a	0.29 ab
Root ad leaf application	7.35 a	1.90 a	0.26 a	407.2 a	0.29 ab	0.37 a
Significance[a]						
Salinity (S)	*	*	ns	ns	ns	*
Biostimulant (B)	**	**	**	**	*	**
S × B	ns	ns	ns	ns	ns	ns

[a] ns. *, ** Nonsignificant or significant at $P \leq 0.05$, and 0.01, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$).

PSII Efficiency

Treatments	SPAD	F_v/F_m	Proline ($\mu g\ g^{-1}$ f. wt.)
Salinity (mM NaCl)			
1	30.65 a	0.82 a	12.1 b
25	26.37 b	0.54 b	26.7 a
Biostimulant			
Control	28.10 a	0.58 b	24.5 a
Root application	28.18 a	0.71 a	17.2 a
Root ad leaf application	29.26 a	0.75 a	16.5 a
Significance[a]			
Salinity (S)	***	***	***
Biostimulant (B)	ns	*	ns
S × B	ns	ns	ns

[a] ns. *, *** Nonsignificant or significant at $P \leq 0.05$, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$).

Treatments	Major elements ($g\ kg^{-1}$)					
	N	P	K	Ca	Mg	Na
Salinity (mM NaCl)						
1	36.2 a	4.1 a	39.4 a	7.9 a	2.4 a	2.7 b
25	32.0 b	3.5 b	30.6 b	5.8 b	1.1 b	13.5 a
Biostimulant						
Control	32.0 b	3.4 b	34.0 a	6.7 a	1.6 a	8.8 a
Root application	35.7 a	3.8 ab	35.4 a	7.0 a	1.7 a	7.7 a
Root ad leaf application	34.6 a	4.1 a	35.6 a	7.1 a	2.0 a	7.9 a
Significance[a]						
Salinity (S)	*	*	***	***	***	***
Biostimulant (B)	**	**	ns	ns	ns	ns
S × B	*	ns	ns	ns	ns	ns

[a] ns. *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$).

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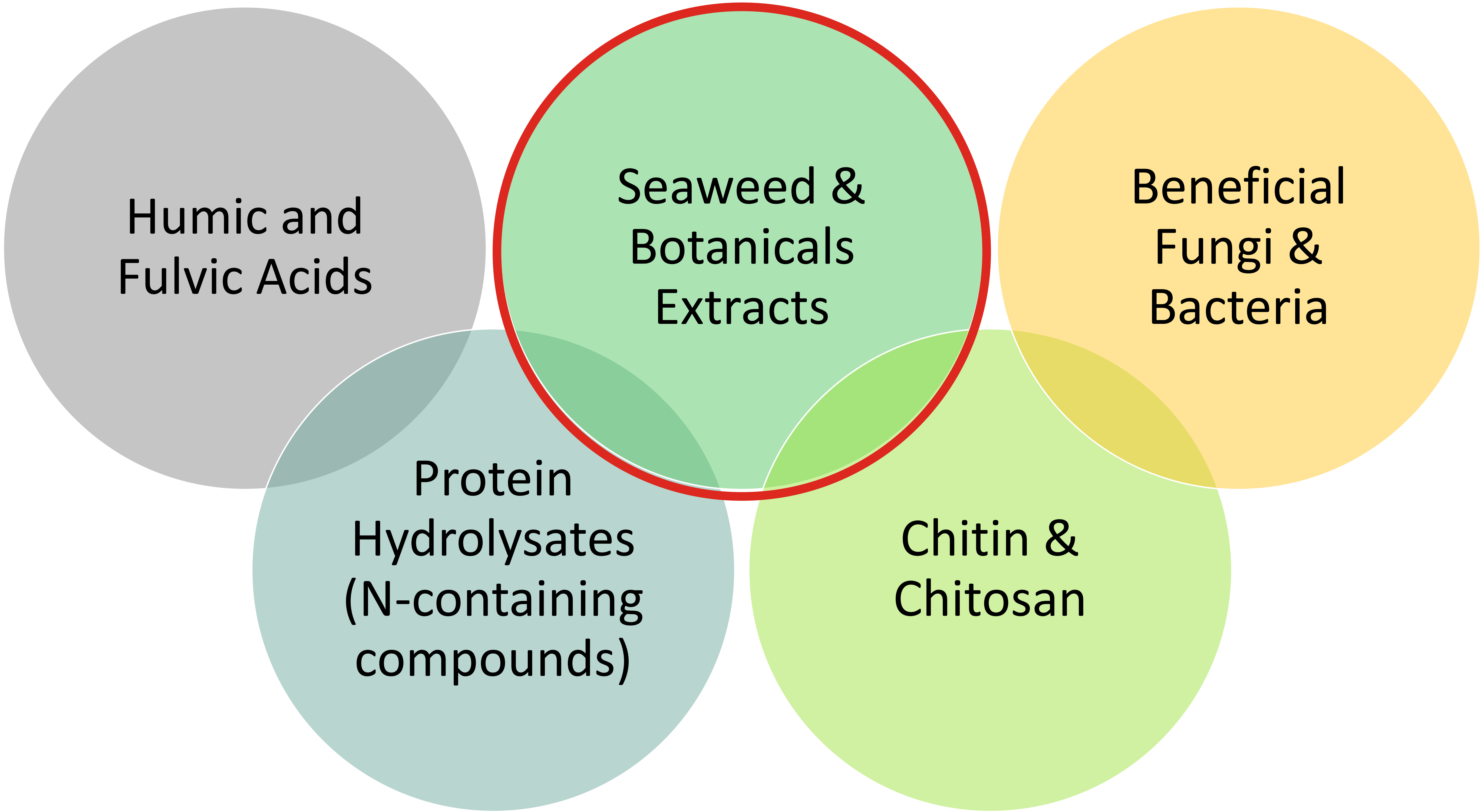
Biostimulant Categories



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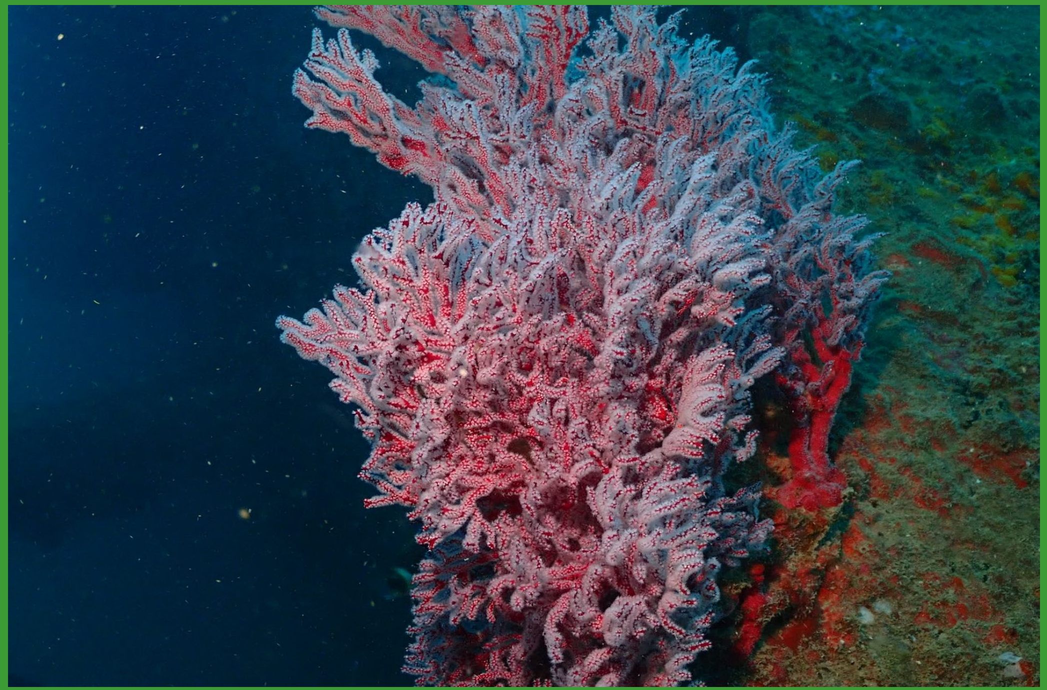
Seaweed & Botanical Extracts

Seaweed = Macroalgae

Ascophyllum nododsum



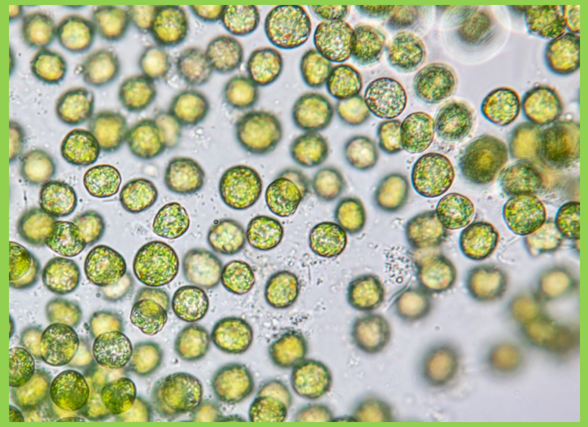
Lithothamnium calcareum

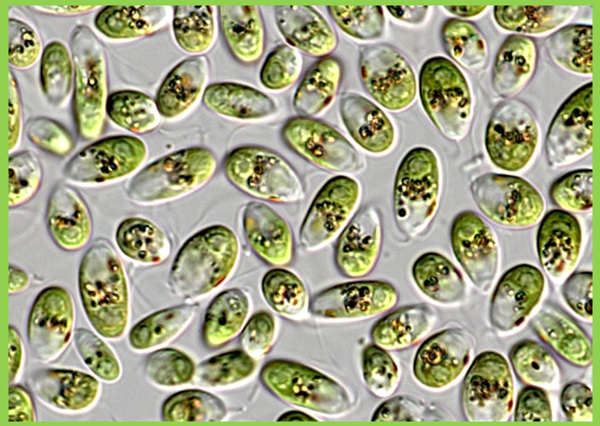
Ecklonia maxima

Codium spp.

Microalgae

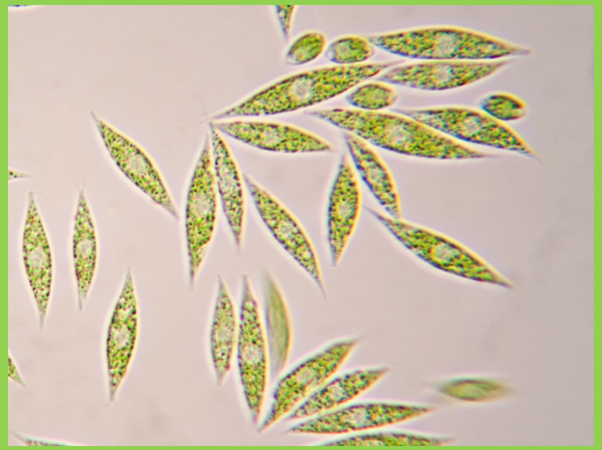
Chlorella spp.





Dunaliella spp.

Euglena gracilis



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Seaweed & Botanical Extracts

Sources of brown (B), green (G) and red (R) seaweed & microalgae (M)

- *Ascophyllum nododsum* (B)
- *Laminaria* spp. (B)
- *Durvillaea potatorum* (B)
- *Ecklonia maxima* (B)
- *Fucus* spp. (B)
- *Macrosystis pyrifera* (B)
- *Lithothamnium calcareum* (R)
- *Kappaphycus alvarezii* (R)
- *Gracilaria debilis* (B)
- *Sargassum cinctum* (B)
- *Codium* spp. (G)
- *Arthrospira* spp. (M)
- *Chlorella* spp. (M)
- *Dunaliella* spp. (M)
- *Nostoc* spp. (M)
- *Aphanizomenon* spp. (M)
- *Euglena gracilis* (M)

Process Methods (Generally Proprietary)

- Extraction with acidified water (pH 3.5), centrifuged to remove excess solids, adjusted to neutral pH
- Cold extraction (alkaline)
- Heat extraction (alkaline)
- Mechanical - homogenization
- Press - filtration
- Fermentation

Components*

- Polysaccharides
- Sterols
- Polyphenolics
- Carotenoids
- Lipids
- Protein, peptides, & amino acids
- Betaines
- Plant growth regulators

***Categorically similar but structural compositions differ by source**

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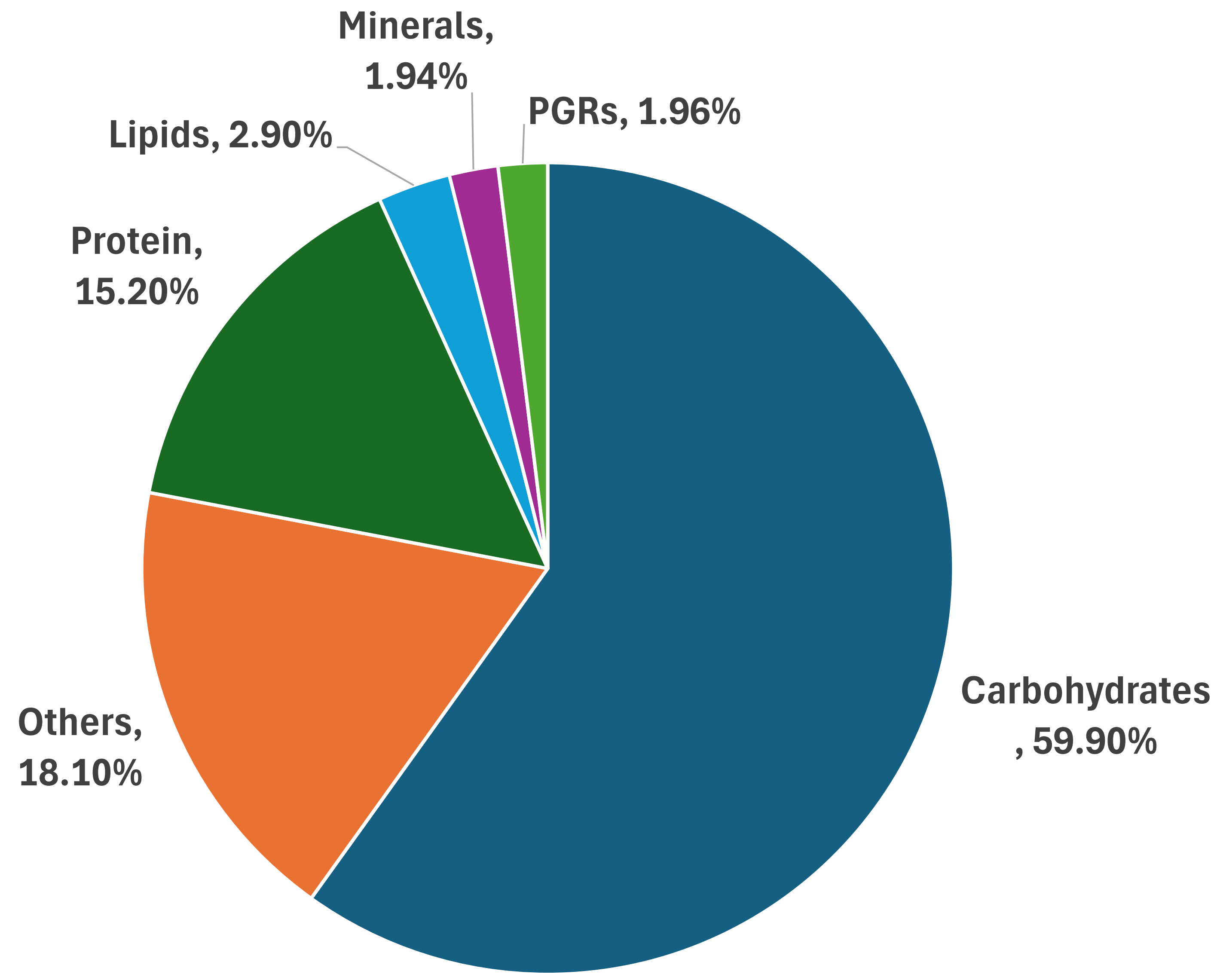
Seaweed & Botanical Extracts

Modes of Action & Function

- **Fertilizers** with micro- and macronutrients
- Polysaccharides contribute to gel formation, water retention and soil aeration
- Polyanionic compounds contribute to the **fixation and exchange of cations**, which is also of interest for the fixation of heavy metals and or soil remediation
- Soil microflora promotion of plant growth-promoting bacteria and pathogen antagonists
- **Up/down regulation of hormonal biosynthetic genes**
    - Impacts on seed germination, plant establishment, growth and development
- Anti-stress effects with antioxidants and **regulators of endogenous stress-responsive genes**



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Seaweed - Artichoke

Spyridon et al. (2022)

Foliar application to drip in 2-week intervals, started 30 days after planting
Mixture of 3 seaweeds: *Ascophyllum nodosum*, *Laminaria* spp., and *Sargassum* sp.

Treatments	First Season (2018–2019)				Second Season (2019–2020)			
Foliar Spraying	Plant Height (cm)	No. of Shoots/Plant	Leaves Fresh Weight/Plant (kg)	Leaves Dry Weight/Plant (g)	Plant Height (cm)	No. of Offshoots/Plant	Leaves Fresh Weight/Plant (kg)	Leaves Dry Weight/Plant (g)
Control	93 ± 1 A	5.7 ± 0.2 C	2.4 ± 0.2 C	404 ± 5 D	97 ± 2 D	5.6 ± 0.2 C	2.5 ± 0.2 B	442 ± 5 C
Seaweed extract 1 g/L	95 ± 1 B	6.5 ± 0.1 B	2.5 ± 0.1 BC	425 ± 2 C	101 ± 2 C	6.1 ± 0.3 B	2.6 ± 0.2 A	476 ± 2 B
Seaweed extract 2 g/L	98 ± 1 C	6.7 ± 0.2 AB	2.6 ± 0.2 AB	450 ± 8 B	107 ± 2 B	6.6 ± 0.2 A	2.7 ± 0.2 A	490 ± 2 AB
Seaweed extract 3 g/L	106 ± 2 D	6.8 ± 0.2 A	2.7 ± 0.2 A	468 ± 3 A	111 ± 1 A	6.6 ± 0.2 A	2.8 ± 0.2 A	507 ± 3 A

Foliar Spraying	N%	P%	K%	Total Carbohydrates %	N%	P%	K%	Total Carbohydrates %
Control	1.8 ± 0.0 D	0.22 ± 0.01 D	1.3 ± 0.0 B	20 ± 1 B	2.1 ± 0.0 C	0.22 ± 0.01 D	1.3 ± 0.0 C	17 ± 1 D
Seaweed extract 1 g/L	2.0 ± 0.0 C	0.23 ± 0.01 C	1.3 ± 0.1 B	20 ± 1 B	2.2 ± 0.1 C	0.23 ± 0.01 C	1.4 ± 0.0 B	19 ± 1 C
Seaweed extract 2 g/L	2.2 ± 0.1 B	0.24 ± 0.01 B	1.5 ± 0.0 A	23 ± 1 A	2.3 ± 0.1 B	0.23 ± 0.01 B	1.6 ± 0.0 A	20 ± 1 B
Seaweed extract 3 g/L	2.4 ± 0.1 A	0.26 ± 0.01 A	1.5 ± 0.1 A	23 ± 1 A	2.4 ± 0.0 A	0.25 ± 0.01 A	1.6 ± 0.1 A	17 ± 1 A

Foliar Spraying	Early Heads/Plant (no)	Late Heads/Plant (no)	Total Heads/Plant (no)	Total Yield (ton/ha)	Early Heads/Plant (no)	Late Heads/Plant (no)	Total Heads/Plant (no)	Total Yield (ton/ha)
Control	3.6 ± 0.2 D	6.6 ± 0.1 D	11 ± 0.2 C	3.3 ± 0.3 D	3.5 ± 0.2 D	6.5 ± 0.1 D	11 ± 0.1 B	3.1 ± 0.2 D
Seaweed extract 1 g/L	4.0 ± 0.2 C	7.7 ± 0.1 C	12 ± 0.2 C	3.8 ± 0.1 C	3.9 ± 0.1 C	7.6 ± 0.1 C	11 ± 0.2 B	3.6 ± 0.2 C
Seaweed extract 2 g/L	4.3 ± 0.2 B	8.3 ± 0.1 B	13 ± 0.1 B	4.5 ± 0.4 B	4.2 ± 0.1 B	8.1 ± 0.1 B	12 ± 0.1 A	4.2 ± 0.2 B
Seaweed extract 3 g/L	4.5 ± 0.2 A	8.9 ± 0.2 A	13 ± 0.2 A	5.0 ± 0.1 A	4.8 ± 0.2 A	8.7 ± 0.2 A	14 ± 0.1 A	4.9 ± 0.2 A

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Seaweed - Artichoke

Spyridon et al. (2022)

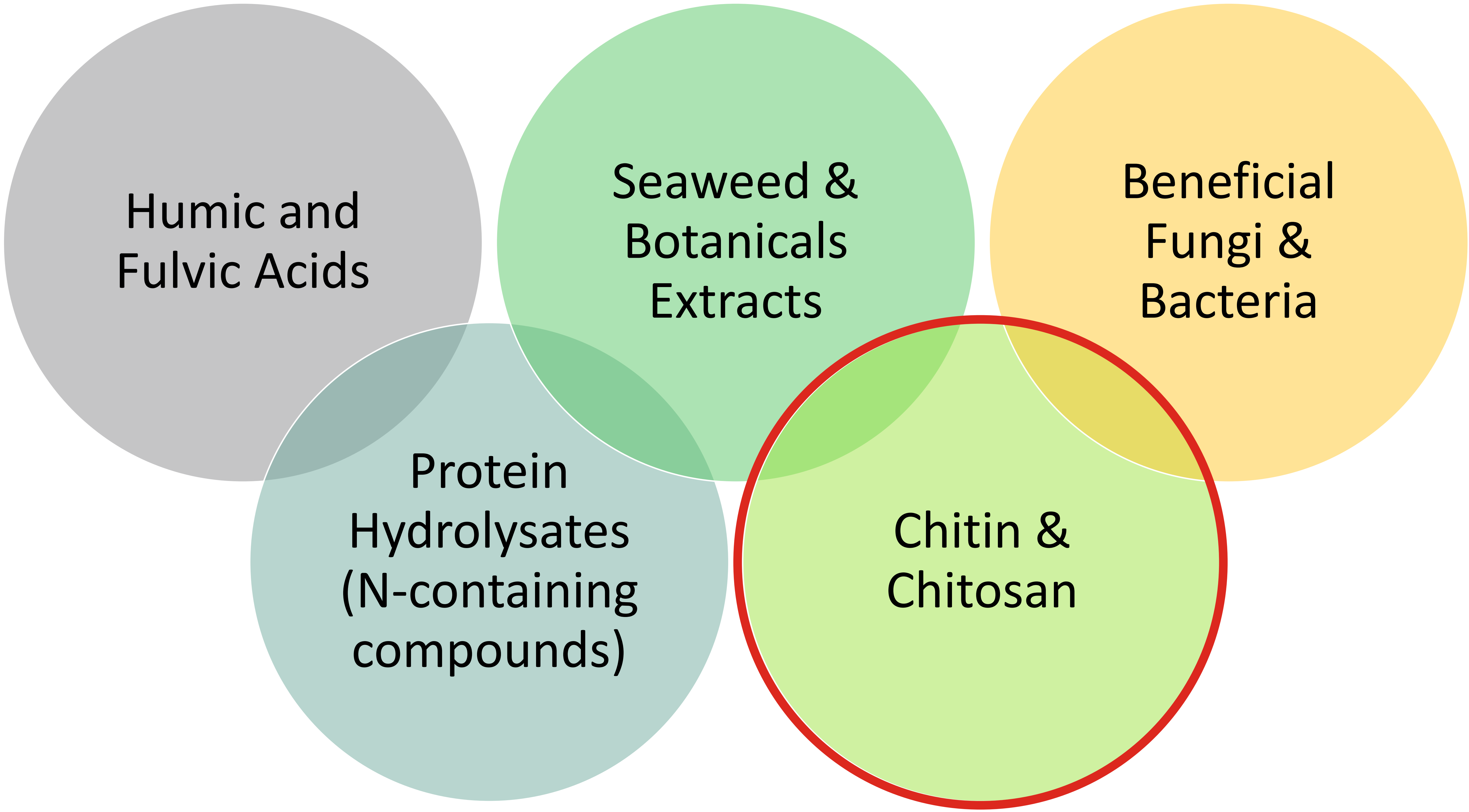
Foliar application to drip in 2-week intervals, started 30 days after planting
Mixture of 3 seaweeds: *Ascophyllum nodosum*, *Laminaria* spp., and *Sargassum* sp.

	First Season (2018–2019)			Second Season (2019–2020)		
Foliar Spraying	Total Flavonoids (mg of Catechin/100 g f.w.)	Fiber (%)	Total Phenolic Content (mg of Gallic Acid/100 g f.w.)	Total Flavonoids (mg of Catechin/100 g f.w.)	Fiber (%)	Total Phenolic Content (mg of Gallic Acid/100 g f.w.)
Control	11 ± 0.3 D	6.9 ± 0.3 A	276 ± 2 D	11 ± 0.3 D	6.7 ± 0.1 A	277 ± 4 D
Seaweed extract 1 g/L	12 ± 0.4 C	6.8 ± 0.1 A	286 ± 4 C	12 ± 0.4 C	6.6 ± 0.1 B	297 ± 8 C
Seaweed extract 2 g/L	13 ± 0.4 B	6.6 ± 0.1 B	301 ± 3 B	13 ± 0.4 B	6.2 ± 0.1 C	314 ± 4 B
Seaweed extract 3 g/L	13 ± 0.3 A	6.1 ± 0.1 C	309 ± 4 A	14 ± 0.4 A	5.6 ± 0.1 D	322 ± 5 A

Foliar Spraying	TSS (%)	Total Sugars (%)	Inulin (%)	TSS (%)	Total Sugars (%)	Inulin (%)
Control	7.2 ± 0.1 C	6.1 ± 0.0 D	1.3 ± 0.0 C	7.3 ± 0.0 C	6.2 ± 0.0 B	1.3 ± 0.1 B
Seaweed extract 1 g/L	7.2 ± 0.0 C	6.2 ± 0.0 C	1.4 ± 0.0 B	7.4 ± 0.1 B	6.3 ± 0.1 B	1.3 ± 0.0 B
Seaweed extract 2 g/L	7.3 ± 0.1 B	6.5 ± 0.1 B	1.4 ± 0.1 A	7.5 ± 0.1 AB	6.4 ± 0.1 A	1.4 ± 0.1 A
Seaweed extract 3 g/L	7.4 ± 0.0 A	6.6 ± 0.1 A	1.5 ± 0.1 A	7.6 ± 0.1 A	6.5 ± 0.1 A	1.4 ± 0.1 A

Foliar Spraying	Head Fresh Weight (g)	Edible Fresh Weight (g)	Head Length (cm)	Head Diameter (cm)	Head Fresh Weight (g)	Edible Fresh Weight (g)	Head Length (cm)	Head Diameter (cm)
Control	175 ± 1 D	51 ± 1 D	9.0 ± 0.1 C	7.4 ± 0.1 D	168 ± 1 D	48 ± 1 D	9.0 ± 0.1 D	7.3 ± 0.1 D
Seaweed extract 1 g/L	187 ± 3 C	55 ± 1 C	9.1 ± 0.2 C	7.6 ± 0.1 C	180 ± 2 C	51 ± 1 C	9.1 ± 0.1 C	7.5 ± 0.1 C
Seaweed extract 2 g/L	201 ± 2 B	43 ± 1 B	9.3 ± 0.2 B	8.0 ± 0.1 B	194 ± 4 B	54 ± 1 B	9.2 ± 0.2 B	7.9 ± 0.1 B
Seaweed extract 3 g/L	210 ± 4 A	64 ± 1 A	9.5 ± 0.1 A	8.1 ± 0.1 A	201 ± 4 A	59 ± 1 A	9.4 ± 0.2 A	8.0 ± 0.1 A

Biostimulant Categories



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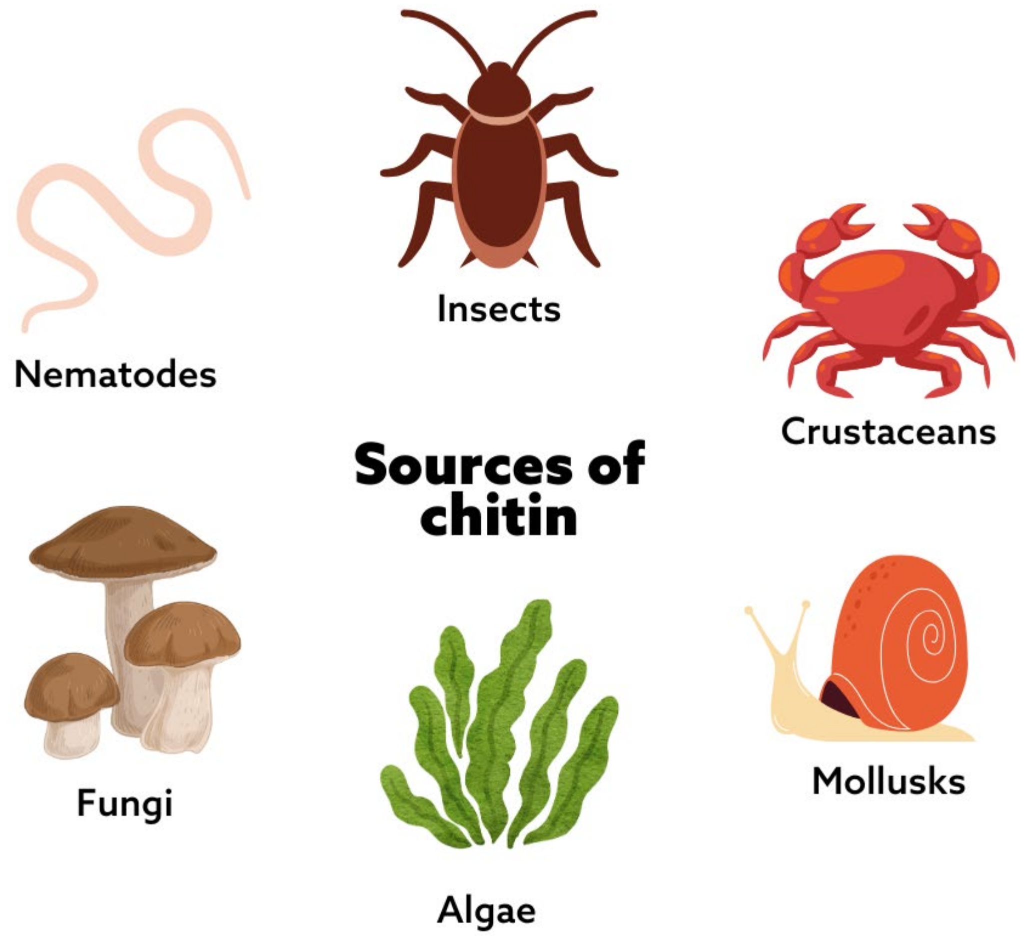
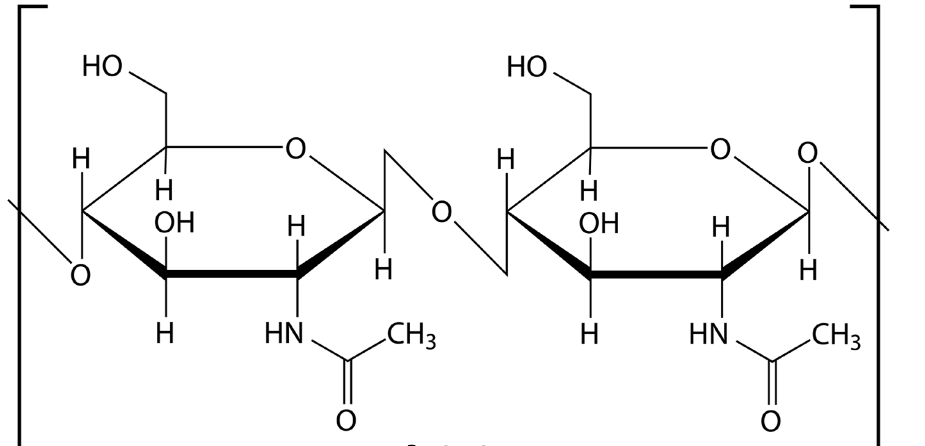
Chitin & Chitosan

- **Chitin** – important structural compound found in the cell walls of crustaceans, fungi, insects, microorganisms, some invertebrate animals and algae
  - Large molecular weight, 1-2.5 x 10^6 Da
  - >50% N-acetyl-glucosamine units
  - Water insoluble
  - Soil amendment

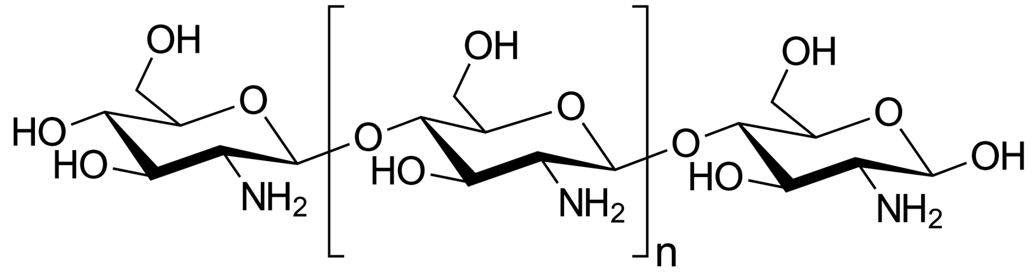
  - **Typical Processing**: grind/mill, demineralization, deproteination, bleaching, deacetylation

- **Chitosan** – derived from chitin, a copolymer of glucosamine and N-acetyl glucosamine
  - Deacetylated chitin
  - ≤50% N-acetyl-glucosamine units
  - Range of molecular weight, 2 x 10^5 to 1 x 10^6 Da
  - Water soluble (acid required if >30 kDa)





Chitin



Chitosan

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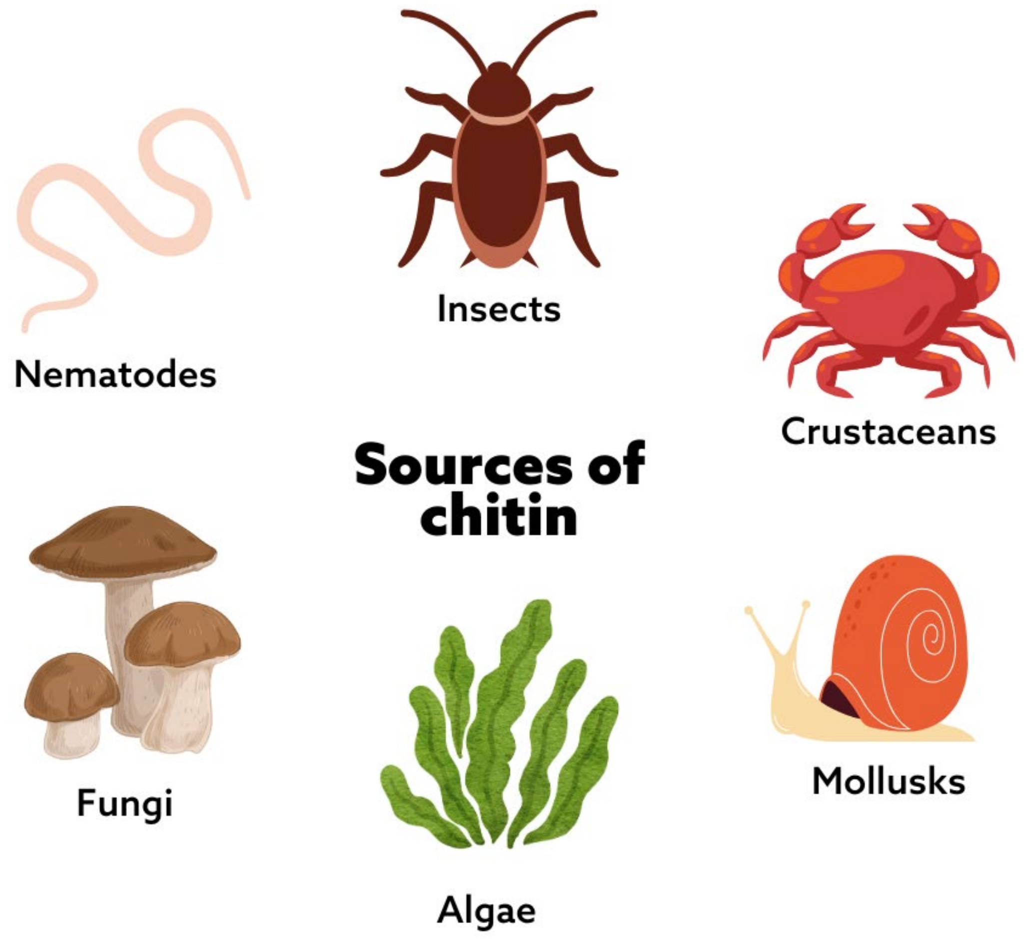
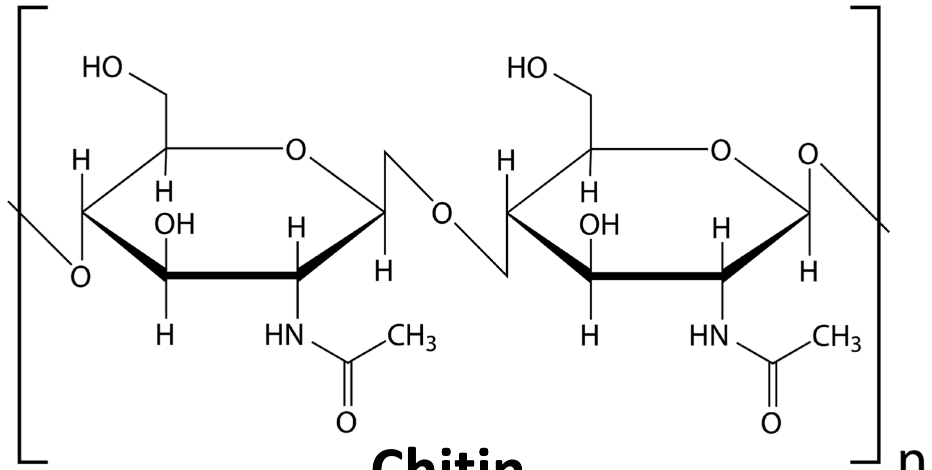
Chitin & Chitosan

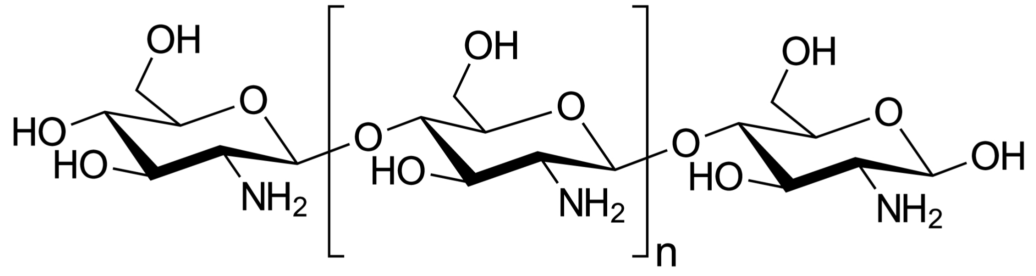
Modes of Action & Function

- Polyelectrolyte & **chelation**
  - Protonated NH_2 group enables complexes with negative charged derivatives (i.e. proteins, dyes, enzymes, bacteria cell wall proteins, DNA, RNA, metals
  - Improves soil properties and prevents nutrient leaching
- **Defense activation** via signaling pathways (increased peroxide accumulation and Ca^{2+} leakage)
- **Antimicrobial** (bacteria, fungi, nematodes)
  - increases membrane permeability and loss of intracellular substances (inhibits growth)
  - Improves shelf life of vegetable products
- Tolerance to abiotic stress
  - Drought (stomatal closure), salinity, cold stress





Chitin



Chitosan

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Chitosan - Oregano

Mohammadi et al. (2021)

- Random complete block design with 3 replications per treatment
- Treatments:
  - 3 rates of chitosan (0, 250, 500 mg/L) applied as foliar spraying, and
  - Drought stress at two levels: well-watered (control) and water deficit stress
  - Two species of origanum (*Origanum vulgare, Origanum majorana*)

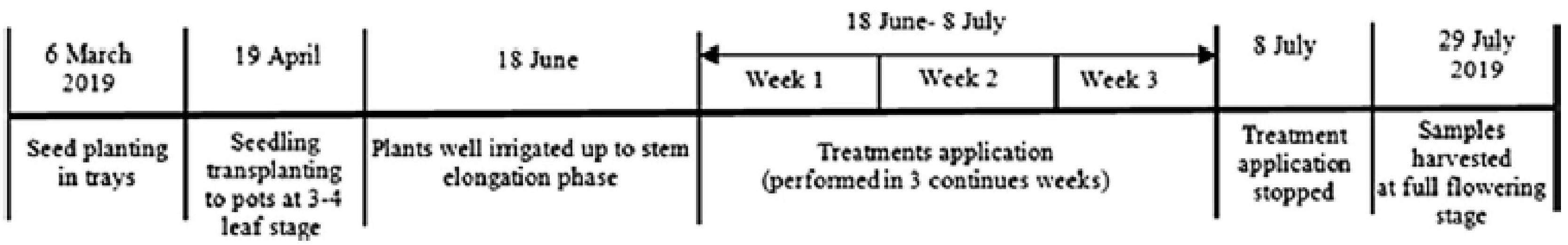
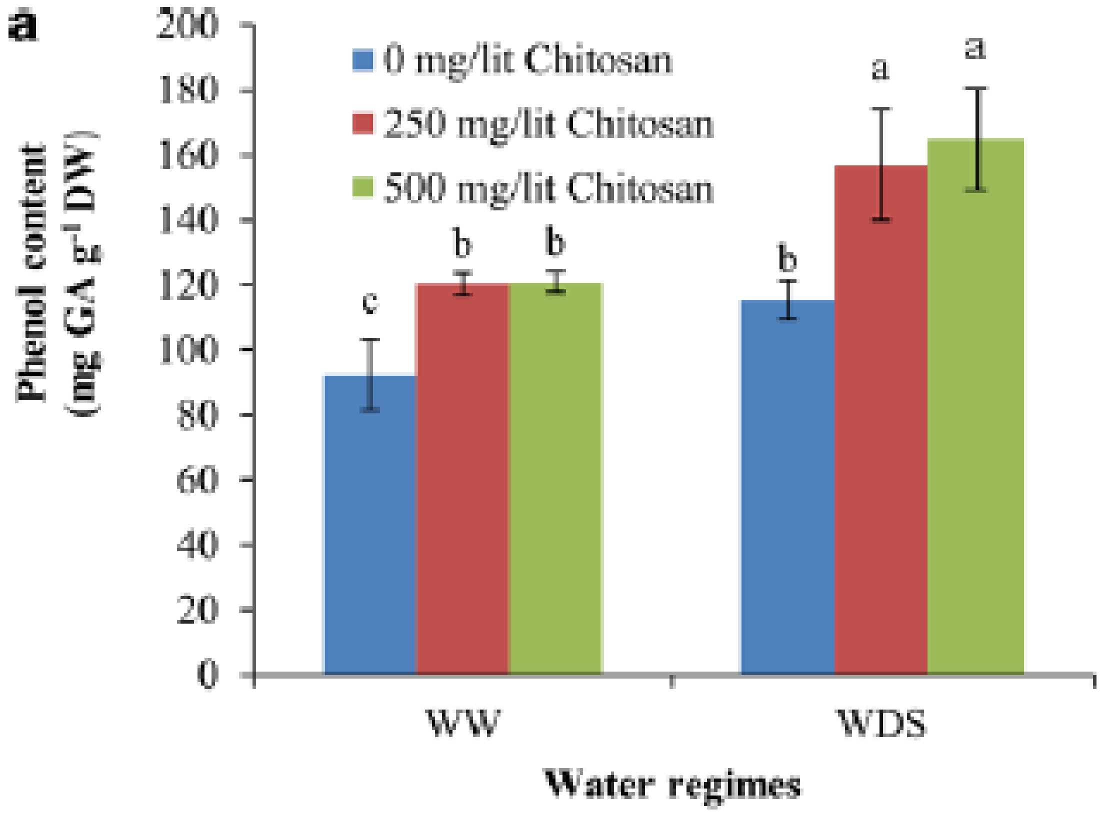


Table 2 Mean comparison of total dry weight and essential oil content in two origanum species exposed to different concentrations of chitosan under well-watered and water deficit stress conditions

Water regimes	Species	Chitosan (mg/L)	Total dry matter (g)	Essential oil content (% w/w)
Well-watered	*O. vulgare*	0	$2.576^{b} \pm 0.194$	$0.309^{cd} \pm 0.025$
		250	$3.297^{a} \pm 0.237$	$0.427^{c} \pm 0.037$
		500	$3.651^{a} \pm 0.155$	$0.396^{c} \pm 0.024$
	O. majo-rana	0	$1.612^{c} \pm 0.024$	$0.319^{cd} \pm 0.015$
		250	$2.770^{b} \pm 0.046$	$0.209^{de} \pm 0.008$
		500	$3.501^{a} \pm 0.206$	$0.161^{e} \pm 0.011$
Water deficit stress	*O. vulgare*	0	$1.376^{c} \pm 0.165$	$0.911^{b} \pm 0.090$
		250	$1.298^{c} \pm 0.029$	$1.111^{a} \pm 0.065$
		500	$2.320^{b} \pm 0.181$	$0.446^{c} \pm 0.044$
	O. majo-rana	0	$1.600^{c} \pm 0.089$	$0.349^{c} \pm 0.015$
		250	$1.664^{c} \pm 0.168$	$0.348^{c} \pm 0.028$
		500	$1.601^{c} \pm 0.030$	$0.360^{c} \pm 0.015$
Alpha value			0.448	0.119

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test ($n = 3$)



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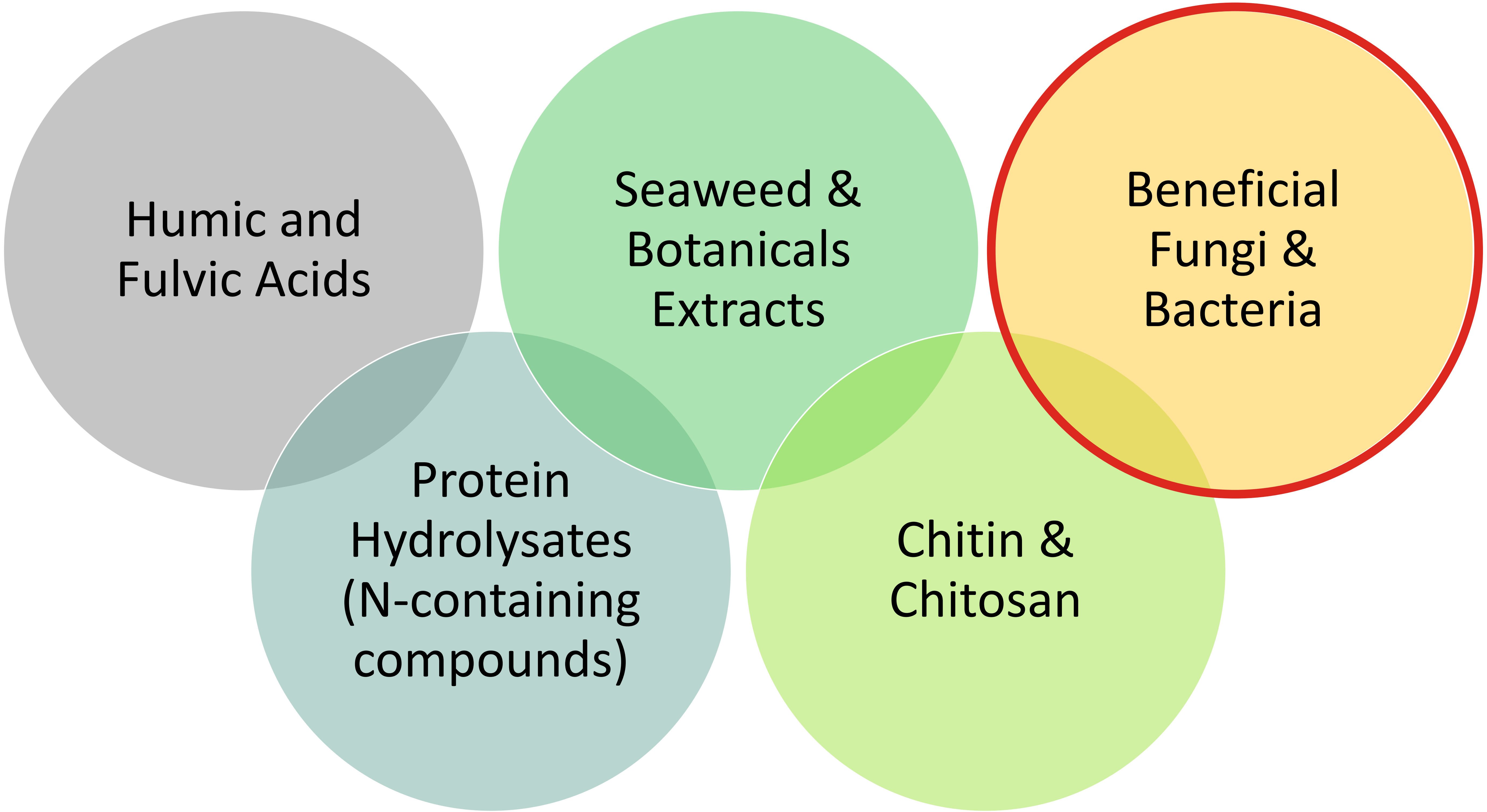
Biostimulant Categories



Beneficial Microorganisms

Co-evolution of mutually beneficial to parasitic relationships with plants

- **Fungi**
  - Mycorrhizal fungi – 90% symbioses of plant taxa
  - Nutritional efficiency – macronutrients (P & N) & micronutrients
  - Arbuscules – increase water and nutritional use efficiency to the roots for increased abiotic stress tolerance

- **Bacteria**
  - Rhizosphere – niches that extend from the soil to the interior of cells of the plant roots
  - Transient or permanent
  - Supply nutrients, increase nutrient use efficiency, induction of disease resistance
  - Enhance abiotic stress tolerance
  - Plant growth promoting rhizobacteria

- Likely that a large portion of the estimated ~45,000 mycorrhizal fungal species are potential biostimulants

- A wide range of bacterial species are considered biostimulants - *Bacillus, Pseudomonas, Azospirillum, Rhizobium, Arthrobacter, Serratia, Enterobacter*

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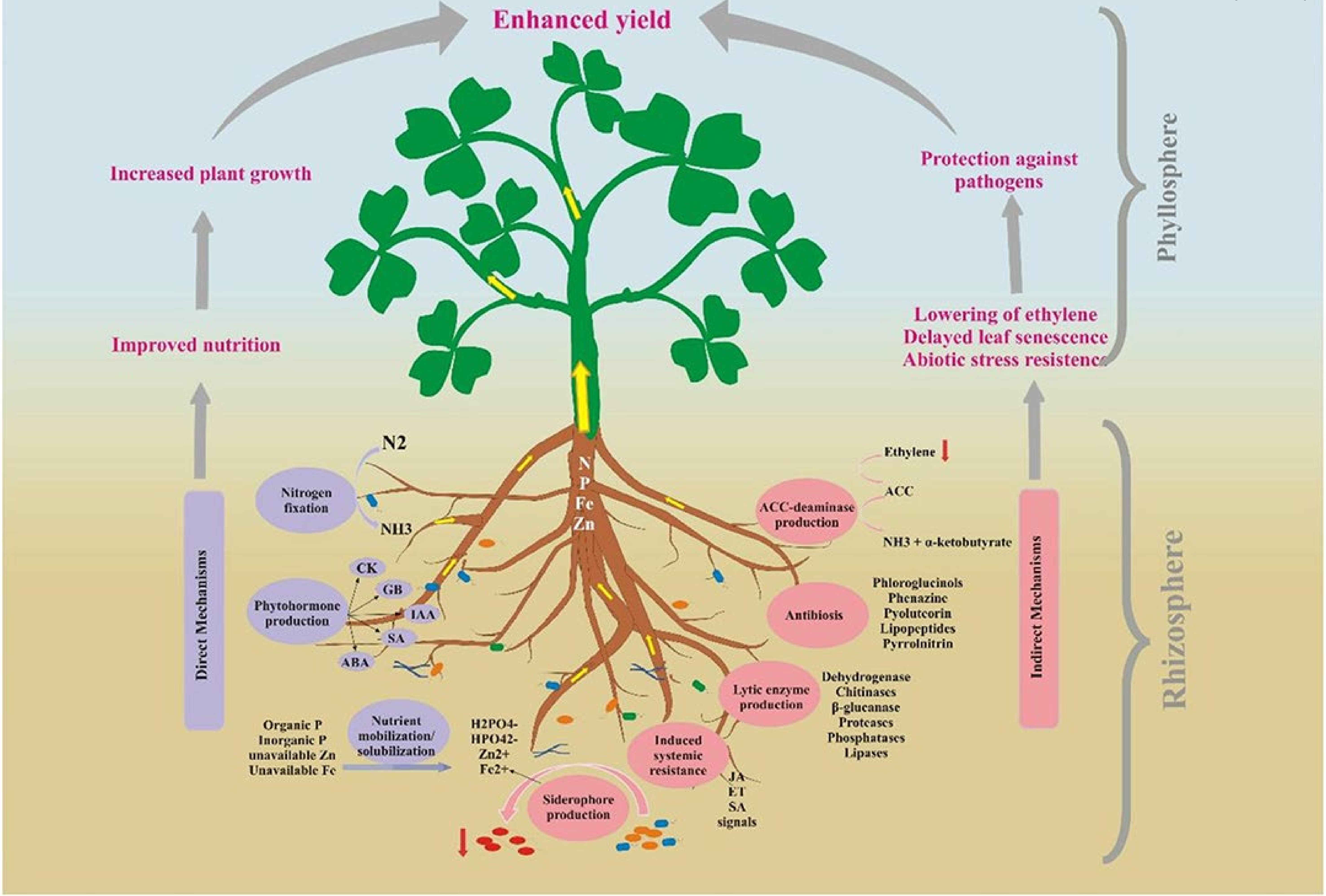
Hakim et al. (2021)



Beneficial Micro - Shallots

Golubkina et al. (2019)

Split plot deign with 3 replicates per treatment
Treatments: factorial combination

- Inoculated and non-inoculated control – applied to hole at bulb planting
- two selenium treatments (63 mg Se/m2 of either selenocystine or sodium selenate), and non-treated control – spray applied 4 and 6 weeks after planting

Innoculum: *Rhizophagus intraradices* (endomycorrhizal fungus), *Trichoderma harzianum*, and *Bacillus subtilis*

Experimental Factor	Mycorrhizal Index	Growth Indices				Marketable Bulbs				
	%	LAI ($m^2 \cdot m^{-2}$)		Dry Matter ($g \cdot m^{-2}$)		Yield ($Mg \cdot ha^{-1}$)		Mean Weight (g)		Number per Plant
Microorganism inoculation										
AMF formulate	65.7 ± 2.6	1.25 ± 0.11		2.91 ± 0.23		54.9 ± 2.4		22.3 ± 1.4		6.0 ± 0.3
Non-inoculated control	26.4 ± 1.1	0.82 ± 0.06		1.92 ± 0.15		37.3 ± 1.7		15.2 ± 0.9		6.1 ± 0.3
	*	*		*		*		*		n.s.
Selenium treatment										
Selenocystine	47.5 ± 1.8	1.18 ± 0.11	a	2.77 ± 0.23	a	52.2 ± 2.1	a	22.3 ± 1.5	a	5.8 ± 0.3
Sodium selenate	46.0 ± 1.5	1.00 ± 0.07	b	2.34 ± 0.16	b	45.0 ± 1.7	b	17.6 ± 1.1	b	6.3 ± 0.4
Non-treated control	44.7 ± 1.6	0.92 ± 0.07	b	2.14 ± 0.13	b	41.2 ± 2.3	b	16.5 ± 1.2	b	6.2 ± 0.3
	n.s.									n.s.

LAI, leaf area index; n.s. not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan's test at $p \leq 0.05$. Abbreviations: LAI, leaf area idex; AMF, arbuscular mycorrhizal fungi.

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Beneficial Micro - Shallot

Hakim et al. (2021)

Split plot deign with 3 replicates per treatment

Treatments: factorial combination

- Inoculated and non-inoculated control – applied to hole at bulb planting
- two selenium treatments (63 mg Se/m2 of either selenocystine or sodium selenate), and non-treated control – spray applied 4 and 6 weeks after planting

Innoculum: *Rhizophagus intraradices* (endomycorrhizal fungus), *Trichoderma harzianum*, and *Bacillus subtilis*

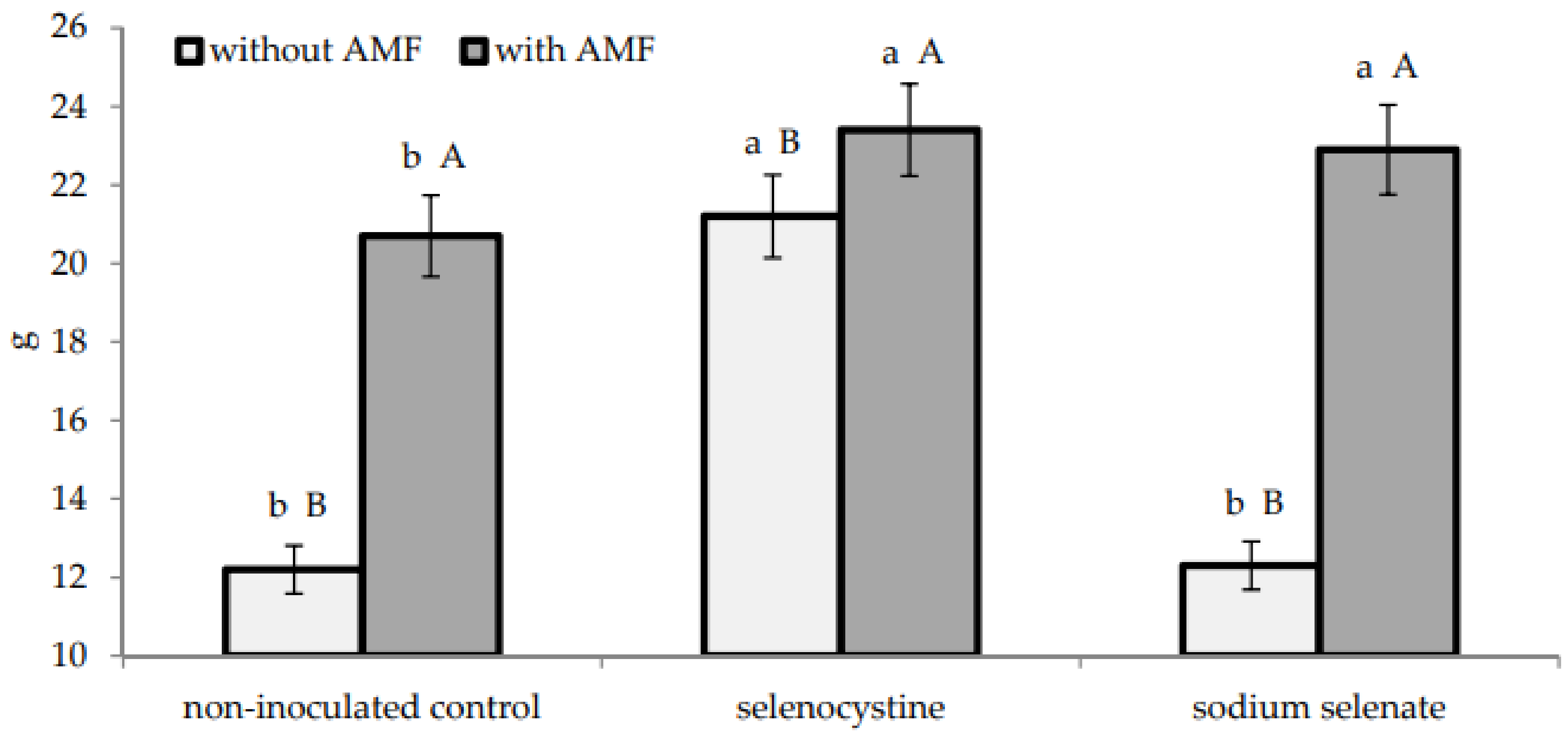

Figure 3. Interaction between microorganism inoculation and selenium treatment on shallot mean bulb weight. The lowercase letters refer to comparison between the selenium treatments within the AMF formulate and control, while capital letters refer to comparison between the AMF formulate and control within each selenium treatment; this is according to Duncan's test at $p \leq 0.05$ with three replicates per treatment.

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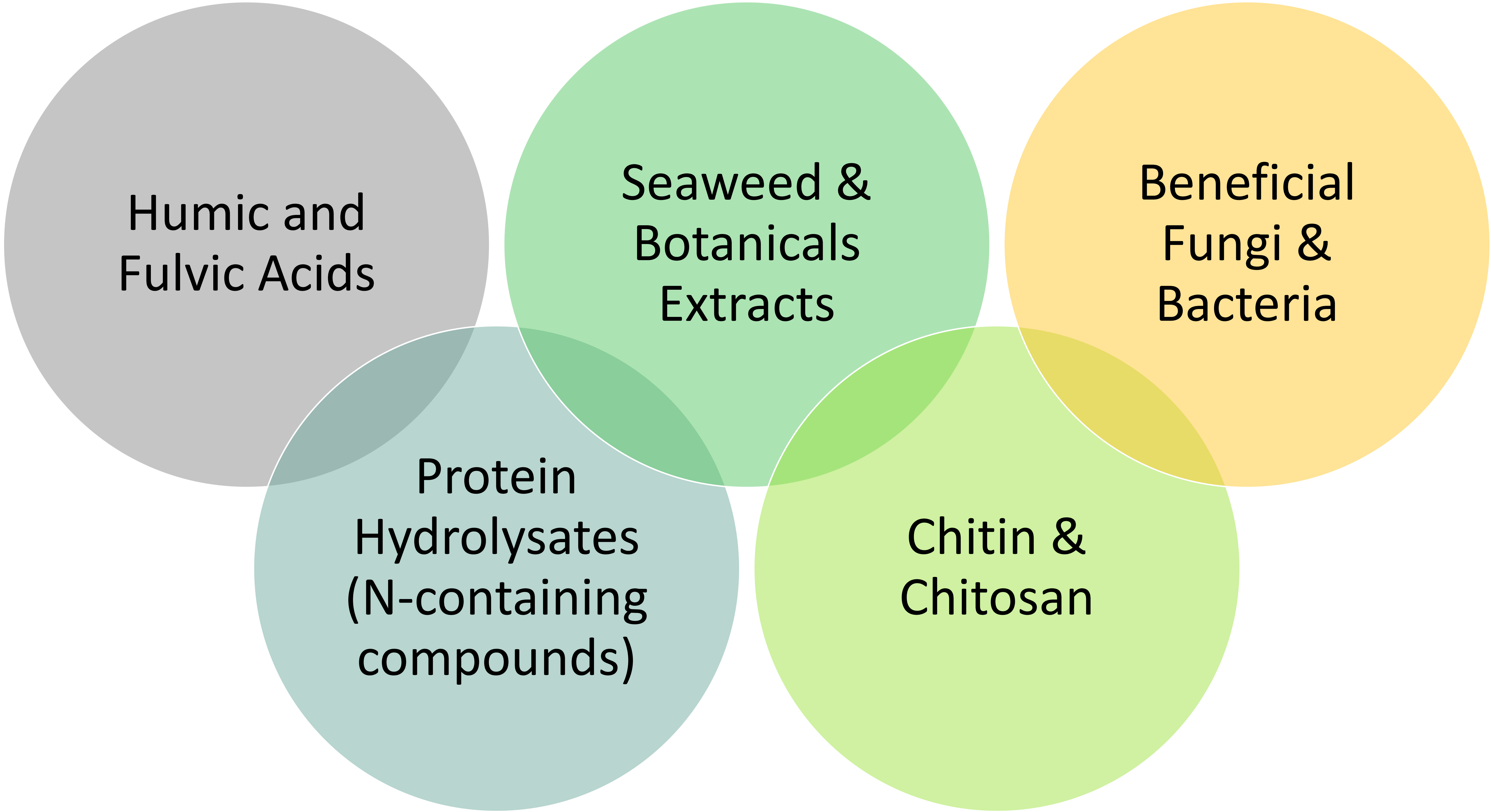
Biostimulant Categories



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Closing thought…

- Biological systems are inherently complex
- Biostimulant product characterizations with replicated and rigorous independent validation is still limited
- There are a significant number of rigorous independent reports of biostimulant benefits on crops
  - Plant metabolism and productivity constraints are not fully understood
- Strong market growth data demonstrates support for biostimulant products within agriculture
- Biostimulants are potentially important if we are to achieve the goal of increased global food production

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