Agricultural applications of seaweed extracts

Seaweeds for plant care: review and experiments in the Netherlands

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Preface

Seaweed is a nutritious and versatile crop that is increasingly important as a healthy and sustainable food source for people and animals. Seaweed production does not require agricultural land or fresh water, and all its biomass can be used. The purpose of ProSeaweed is to create a sustainable source of healthy food products, additives and feed by means of cultivation in the Dutch waters.

The Dutch ministry has commissioned Wageningen Research to design a Research and Development Program to focus on the applicability of seaweed for food and feed. The program must address the following questions:

- Can seaweed become an alternative sustainable resource for protein-from-soya?
- What are food safety aspects of seaweed when it is used as food or feed?
- What are the effects of seaweed cultivation in the marine environment?
- How can seaweed cultivation become a viable business chain?

This report is part of WP 3.5: Impact of seaweed extracts on plant nutrition and plant health. The company Olmix was partner in this research.

Summary

Plant biostimulants are products that have a growth-promoting and/or stress-reducing effect on agricultural crops by improving the condition or resistance of the plant. This allows e.g. better absorption of nutrients, reduced susceptibility to abiotic stresses such as drought, and better plant defences against biotic stresses such as fungal or bacterial attack. Extracts from various seaweed species may have such a biostimulating effect. In this project, published literature on research into the biostimulative effects of various seaweed species on crops and various (probable) candidate substances in seaweed that cause this effect was reviewed. Greenhouse and field experiments were then carried out to examine the effects of seaweed products produced by the French company Olmix on tomatoes, onions and potatoes affected by the fungus *Phytophthora infestans* (the cause of e.g. potato blight). The biostimulant effect of a self-produced extract of the seaweed species *Saccharina latissima* was tested in a small additional trial. *Saccharina* is a seaweed species that occurs naturally in the North Sea and is currently one of the most commonly used species in commercial cultivation of seaweed in European waters.

Olmix's commercial seaweed product (Seamel) is produced from green and red seaweeds harvested in the wild off the Atlantic coast of France. In addition to seaweed, full-formulation Seamel, which is designed for use in crop production, contains various micro- and macro-elements added to enhance the effect of the seaweed extract. Field and greenhouse experiments on potatoes and tomatoes affected by *P. infestans* confirmed the biostimulating effect of the seaweed product, with crops sprayed with Seamel suffering significantly less late blight infection. A dose effect in f.e. the pot trial in tomato in 2018 was also observed, with a higher Seamel dose giving a stronger reduction or delay in infection in field experiments on potatoes in 2019 and 2021. In field experiments on onions in 2018 and 2019, no effect on yield or storage quality was observed, possibly because there was little natural fungal pressure in those years. In 2020, no significant effect of Seamel application was found for potatoes, because of a very early severe infection early in the season with hardly product with to lees product applied. In a greenhouse experiment in 2021, tomato plants were sprayed with Seamel and then actively infected with *Phytophthora*. In addition to a treatment with the complete Seamel product (full formulation), there was also a treatment with Seamel without additives and with its own extract of *S. latissima.* The full-formulation Seamel product had a positive effect on the infected tomato plants, but the product without additives had no significant effect. Biochemical analyses indicated that the latter product was probably made from a different seaweed batch, which may partly explain the lack of effect. Thus further studies are needed on different doses of the additives, alone and in combination with the seaweed in the full-formulation Seamel product, using one seaweed batch for all treatments. The extract of *S. latissima* without additives also had no reducing or delaying effect on *Phytophthora* infection. However, this treatment was only included once in the experiments, so more research is needed to enable firm conclusions to be drawn about its biostimulant effect.

Use of seaweed products with a biostimulant effect offers promising opportunities, e.g. in organic agriculture, but more research is needed to identify the active ingredient(s) and assure high quality, guaranteed function and stability of such products made from wild-harvested seaweed.

1 Effects of seaweed products on plants and/or soils

1.1 General: Seaweeds as biostimulants

1.1.1 General definition of biostimulant

“Biostimulants” (plant growth promoters) is a collective term for a wide range of substances and/or microorganisms that improve plant productivity or quality through improved nutrient uptake, nutrient use efficiency, and tolerance to biotic and abiotic stress when applied to plants or the rhizosphere ((Brown and Saa 2015); (Povero et al. 2016) quoting European Biostimulant Industry Council [EBIC], 2016;(Yakhin et al. 2017)). The definition of biostimulants explicitly excludes known plant nutrients, plant growth regulators, or plant protective compounds. Reasons are that on the one hand producers want a clear distinction from existing legislative product categories ((Yakhin et al. 2017)) in order to avoid exhaustive and expensive safety and efficacy testing and product registration and, and on the other hand, to highlight the fact that much of the functioning of biostimulants is based on mechanism(s) different from those known for fertilizers, plant hormones or plant protective compounds. (du Jardin 2015) came to the following definition: “A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content”. In addition, they stated that “by extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms”. They summarize that ‘biostimulant’ is a versatile term for any substance beneficial to plants with-out being nutrients, pesticides, or soil improvers.

1.1.2 Legislation: New EU Fertilising Products Regulation

Recently (July 15th 2019), the new EU Fertilising Products Regulation (FPR) (EU) 2019/1009 (EU, 2019) has entered into force, recognizing plant biostimulants as a distinct category of agricultural inputs (Chatzikonstantinou, 2019). The regulation will apply from 16 July 16th 2022. Before, biostimulants were at the border between fertiliser and plant protection products. Biostimulants will be excluded from the scope of EU regulation 1107/2009 (EU, 2009), which regulates plant protection products. In the regulation plant biostimulants are defined as follows: *a product stimulating plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits and availability of confined nutrients in soil or rhizosphere*. Explicitly included are micro-organisms that provide the same functions as substances or mixtures. Explicitly excluded from the definition are certain substances, mixtures and micro-organisms that (directly or indirectly) provide protection against pests and diseases, including plant growth hormones and plant growth regulators. These are still classified as Plant Protection Products (PPP) and are subject to the appropriate registration procedures. Biostimulants will be in Category 6 of the seven new Product Function Categories (PFCs) defined by the regulation. Category 6 is divided in two sections: microbial and non-microbial plant biostimulants.
Biostimulants are not allowed to contain certain contaminants above the following concentrations:
(a) Cadmium (Cd): 1,5 mg/kg dry matter
(b) Hexavalent chromium (Cr VI): 2 mg/kg dry matter
(c) Lead (Pb): 120 mg/kg dry matter
(d) Mercury (Hg): 1 mg/kg dry matter
(e) Nickel (Ni): 50 mg/kg dry matter, and

(f) Inorganic arsenic (As): 40 mg/kg dry matter
(g) Copper (Cu): 600 mg/kg dry matter
(h) Zinc (Zn): 1500 mg/kg dry matter
These limits are identical to those for biofertilizers.
Metal concentrations in seaweeds, for example cadmium, are known to sometimes exceed these limits (Lähteenmäki-Uutela et al. 2021). They also write that biostimulants require authorization, and that a positive EU list of accepted biostimulants will be made. The labels of biostimulants are only allowed to contain scientifically proven claims. Work is being done on developing standards for this.

1.1.3 Seaweed product categories

According to Boukhari et al (2020) seaweeds are the dominant category of biostimulants. Products made of seaweeds can be categorized on the basis of the legislative type of product (e.g., PPP or biostimulant), mode of action, composition, function, or mechanism(s). Seaweeds constitute of a mixture of substances, each with a distinct function and possibly with interactions (neutral, synergistic or antagonistic) between functions. For some substances or mechanisms considerably more knowledge is available than for others.

Within the project Bio4safe (WP1) an inventory was made of biostimulant properties of seaweeds (Noordzeeboerderij, 2018a, 2018b). In this inventory the following definition of biostimulants was adapted from (du Jardin 2015): ' any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and /or crop quality traits, regardless of its nutrients content. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and /or microorganisms. Biostimulants are by definition not: fertilizers/nutrients, pesticides, nor soil improvers.' Noordzeeboerderij (2018a) compiled a list of applications in the following areas: agriculture, horticulture, ornamentals and other applications.

They stated that it is not clearly described in the sources they investigated whether biostimulants are effective in either of these industries but they are nevertheless used and assumed to be effective. Seven biostimulant categories are discerned based on (du Jardin 2015): humic/fulvic acids, protein hydrolysates and other N-containing substances, seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds, beneficial fungi and beneficial bacteria. Seaweeds have since ancient times been used as source of nutrients and organic matter, but contain also specific (biostimulant) components: polysaccharides such as laminarin, alginates, ulvans and carrageenans and their breakdown products, micro- and macronutrients, sterols, N-containing compounds like betaines, and hormones.

Seaweeds used as biostimulants are often part of marine macroalgae classes red (Rhodophyta), brown (Phaeophyceae) and green (Chlorophyta) seaweeds.

Table 1 *Overview of seaweeds mentioned as biostimulants (Battacharyya et al, 2015. Noordzeeboerderij, 2018b, Ali et al, 2021)*

Seaweed class	Species
Brown seaweeds	*Ascophylum nodosum*
	Cystoseira myriophylloides
	Ecklonia maxima
	Durvillea potatorum
	Durvillea antartica
	Fucus spp
	Himanthalia elongate
	Hydroclathrus spp.
	Laminaria digitata
	Laminaria hyperborean#
	Macrocystis pyrifera
	Padina pavonica
	Ralfsia spp
	Sargassum species
Green seaweeds	*Ulva spp (Ulva lactuca e.a.)*
	Caulerpa spp
	Codium spp.
	Enteromorpha prolifera
Red seaweeds	*Acanthophora spicifera*
	Ceramium rubrum
	Chondrus crispus
	Cyanidium caldarium
	Gracilaria spp
	Grateloupia turuturu
	Kappaphycus alvarezii
	Laurencia johnstonii
	Macrocycstis pyrifera
	Nereocystis spp.
	Porphyra spp (synonym Pyropia)
	Palmaria palmata
	Soliera cordalis

According to Noordzeeboerderij (2018b) these are the main species used in the biostimulant market (mostly harvested from the wild). Of these five species *Ascophyllum nodossum* is used by 90 % of the interviewed biostimulant companies.

According to (Battacharyya et al. 2015) there are five effects of seaweeds on plants (with examples in seaweeds between brackets): promoting plant growth (plant hormones such as auxins, cytokinins and gibberellins), improving availability of soil nutrients (alginates, fucoidans) and plant nutrient uptake (vitamin K1 derivative), improving coping with abiotic stress (cytokinins), improving plant metabolism and promoting plant health.

1.1.4 Visual overviews of seaweed products for plant growth

Several authors have summarized/visualized the different treatment/application modes, (physiological) effects/benefits and possible mechanisms of seaweed products for plant growth (*Figure 1*, *Figure 2* & *Figure 3*).

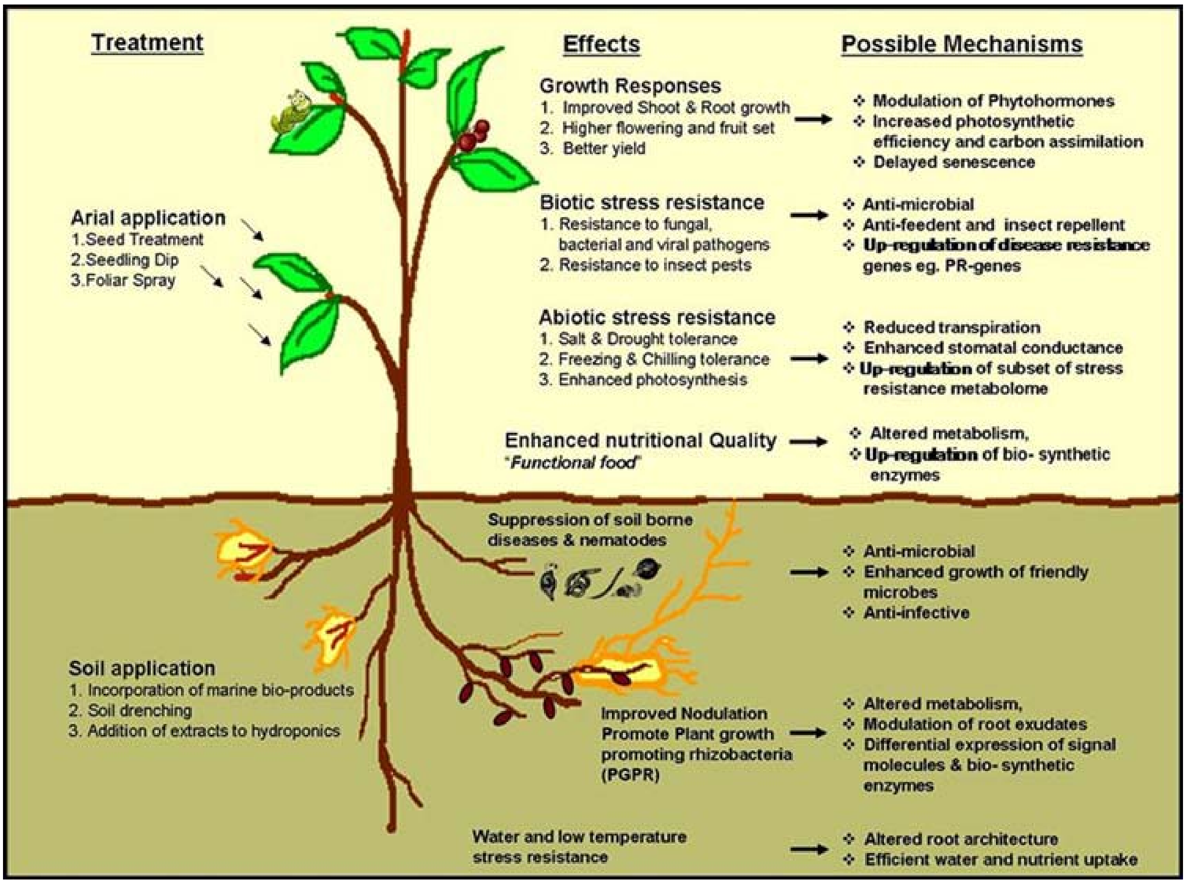

Figure 1 *From: (Khan et al. 2009) Physiological effects elicited by seaweed extracts and Possible bioactivity mechanism(s).*

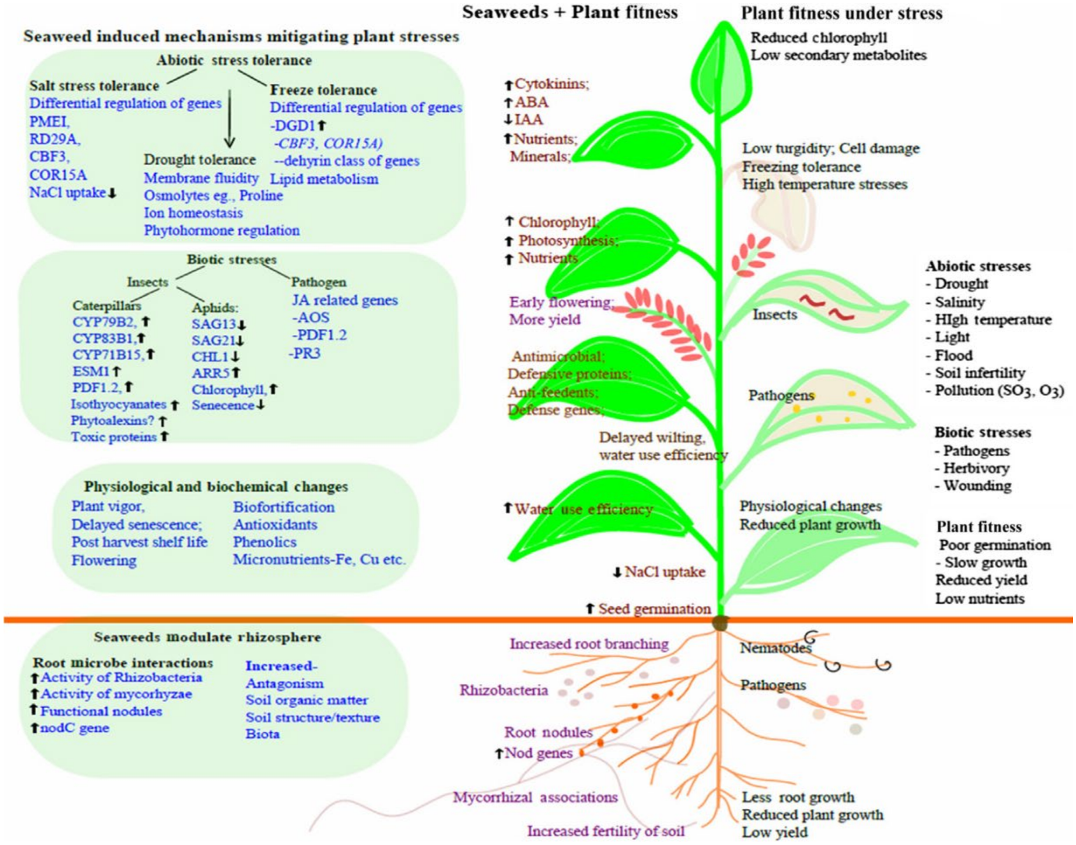

Figure 2 *From: (Sangha et al. 2014) Effects and mechanisms of seaweed extract activities.*

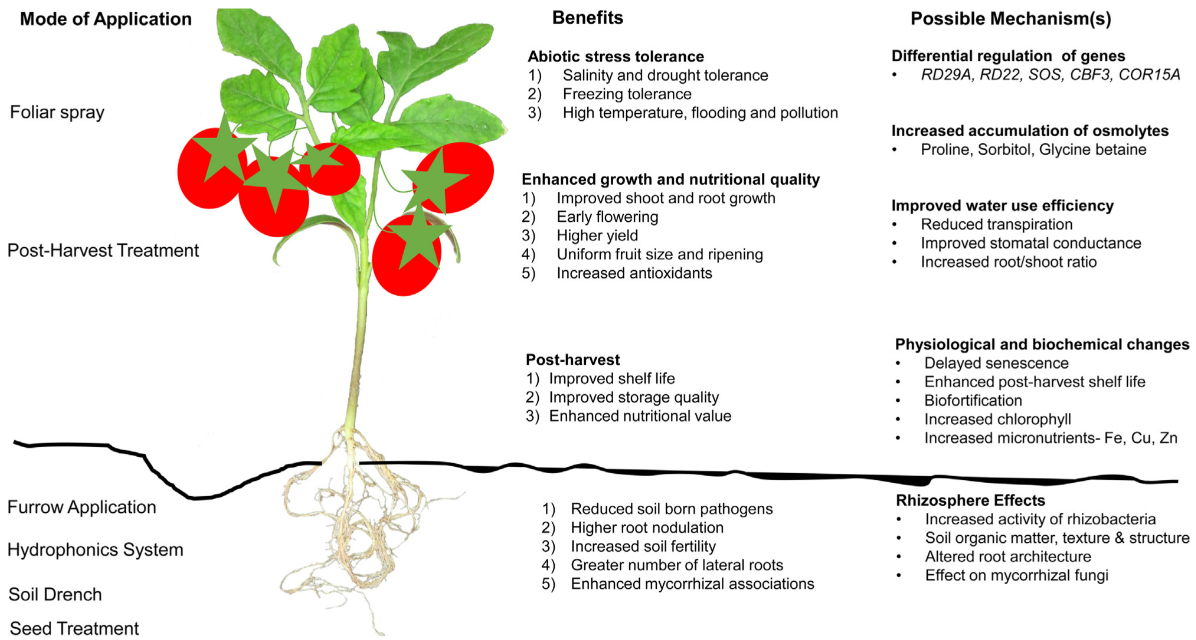

Figure 3 *From: (Battacharyya et al. 2015) Methods of application of seaweed extracts, effects on plants and action mechanisms.*

1.2 Chemical components in seaweeds responsible for plant and soil effects

Biological active compounds in seaweeds can be subdivided into different classes. Stichting Noordzeeboerderij (2018b) described for example the following classes, based on (Yakhin et al. 2017), (Chojnacka et al. 2012), (Bulgari et al. 2015), Pal et al, 2014, (Tuhy et al. 2013):

Table 2 *Major groups of biological active compounds in seaweeds (From: Stichting Noordzeeboerderij, 2018b).*

Group number	Group name	Specific substances	Mode of action
1	Plant growth hormones	Auxins, cytokinins, gibberellins, betaines	Initiate root formation, initiate seeds germination, antiaging, enhances growth, enhances development of flowers and fruits. Enhances nutrient accumulation, stimulates shoot elongation, increases efficiency of water uptake. Effective to reduce effects of abiotic stress e.g. water-, drought- and salt stress
2	Polysaccharides	Galactans, fucoidan, laminarin, alginates	Growth promoting, health improving, antiviral, antimicrobial, antifungal, antioxidant
3	Minerals and vitamins	K, Mg, Ca, Cu, Mn, Fe, I	Essential for plant life cycle, increases crop quality and crop yield
4	Pigments	Carotenoids	Protection from chlorophyll degradation and antioxidant
5	Polyphenols	Tannins, flavonoids	Antibacterial, deterrence of herbivores, protection from UV, release and suppression of growth hormones
6	Proteins	Lectins	Essential source for amino acid formation, increase in biosynthesis, increase carbohydrate concentration in leaves, antimicrobial, antiviral

However, other authors discern different classes, with some overlaps, which makes it sometimes difficult to assign each compound to a class. For this report, the compounds were categorized mainly based on the classes described by (Arioli et al. 2015) and (Khan et al. 2009).

An overview was made of the effects of different seaweed extracts (Appendix I) based on the following six classes:

1. Plant growth regulators (plant hormones/phytohormones)
2. Quaternary ammonium and tertiary sulphonium molecules (osmo-protectants), N-containing compounds
3. Alginate and several polysaccharides (or glycans), some sulphated, and their breakdown products
4. Micronutrients (e.g. minerals, trace elements)
5. Lipid based molecules
6. Secondary metabolites

(Michalak et al. 2020) wrote an extensive review on seaweed extracts as plant biostimulants in agriculture and showed an overview table of research done on the plant promoting effects of seaweeds (biostimulant effects). They also showed an overview table of antifungal effects (PPP effects) and an overview table of effects on plant physiology.

In the following paragraphs the results of Appendix I are summarized. Although the effect of the different components is stated here often these components are influencing each other. Ali et al (2021) states that fractionation of seaweed extracts into their components and their respective bioassays, however, has in their experience not yielded favorable growth effects. Only the whole seaweed extracts have been

consistently proven to be very effective, which highlights the role of multiple components and their complex interactive effects on plant growth processes.
NOTE: references were not checked for the reliability of the presented results, the following paragraphs are mainly a broad overview of what effects of seaweed(s) (components) are summed up in literature. Also, it is hard to prove a direct link between a certain component in a seaweed extract and a specific effect in plants, so care should be taken with the interpretation.
Effects mentioned in this review cannot interpreted standalone without consulting the original sources.

1.3 Plant growth regulators (plant hormones/phytohormones)

Plant growth regulators are for example auxins, cytokinins, ethylene, gibberellins, brassinosterioids and abscisic acid (Arioli et al. 2015) (Khan et al. 2009). In Appendix I a table with researches on these components in seaweeds is shown (Type 1). (Górka and Wieczorek 2017) describe that plant hormones are usually categorized in five classes: auxins, cytokinins, gibberellins, jasmonates and brassinosteroids. These components are responsible for multipe processes during the life cycle of plants: cell division, seed germination, flowering, senescence etc. In a mixture of Baltic Sea algae (e.g. *Cladophora*, *Ulva* and *Polisiphonia*) the authors found trans-zeatin (TZ) and phenylacetic acid (PAA). (Nabti et al. 2017) mention that it is well known that gibberellic acid (GA3) stimulates
seed germination in several plant species by inducing enzymes. (Craigie 2011) mentions that jasmonates from *Fucus* applied on terrestrial plants induce amongst other things defense and stress responses, synthesis of proteinase inhibitors and promote tuber formation.

1.4 Quaternary ammonium and tertiary sulphonium molecules (osmo-protectants), N-containing compounds

Betaines and proline are examples of quaternary ammonium molecules (Arioli et al. 2015). They are involved in protection against osmotic changes and accumulate when stress tolerance against drought or salt increases (Calvo et al. 2014) (Khan et al. 2009). Betaines are also associated with increased chlorophyll content (Khan et al. 2009). In Appendix I a table with researches on these components in seaweeds is shown (Type 2). Also N-containing components like amino acids, proteins and enzymes have been included in the table. (Nabti et al. 2017) mention that betaines have an osmoprotective function. (Blunden et al. 2010) found that betaines lead to higher levels of chlorophyll in treated plants (e.g. dwarf French bean, tomato, wheat, barley, and maize). They also mention reduced nematode invasions in tomato plants. (Spinelli et al. 2010) also suggested that betaines in the extract they used led to an increase in chlorophyll in strawberries. (Roussos et al. 2009) mention that glycine betaine enhanced biosynthesis of some phenolic compounds in strawberry leaves.

1.5 Alginate and several polysaccharides (or glycans), some sulphated, and their breakdown products

Seaweeds can contain unusual and complex polysaccharides, sometimes sulphated, such as laminaran, fucoidan, and alginate (Khan et al. 2009). They have listed 10 polysaccharide components for Chlorophyceae (green seaweeds), 8 for Rhodophyceae (red seaweeds) and 8 for Phaeophyceae (brown seaweeds). In Appendix I a table with researches on these components in seaweeds is shown (Type 3).

The most studied polysaccharides in seaweeds are carrageenans, fucans, laminarans and ulvans (Stadnik and Freitas 2014a). For example, in the article of (Bulgari et al. 2015) it is described how *Arabidopsis* plants treated with carrageenan had a higher tolerance to the fungus *Sclerotinia scleortiorum*, probably by activation of certain genes. (Mercier et al. 2001) describe that carrageenans induced signalling and defence gene expression in tobacco leaves. (Aziz et al. 2003) did research on grapevines in which laminarin was found to induce defense responses and could be used to protect the plants against pathogens like *Botrytis cinerea*. (Mzibra et al. 2018) found that different polysaccharides increased seed germination percentage, plant biomass, as well as chlorophyll content of tomato. (Castellanos-Barriga et al. 2017) mention research that finds that both polysaccharides such as ulvans and oligosaccharides can be used as biological plant protection agent.

1.6 Micronutrients (e.g. minerals, trace elements)

Minerals and trace elements in seaweeds can be a nutritive source or have a role in plant development (Arioli et al. 2015). Examples are manganese, calcium and sodium. In Appendix I a table with researches on these components in seaweeds is shown (Type 4). In (Sivasankari et al. 2006) it is mentioned that the micro and macronutrients in seaweeds could be responsible for plant growth enhancement. (Bikker et al. 2016) however mention that high mineral contents (e.g. Na, Cl, K) of seaweeds may also have adverse effects. Based on (Colla et al. 2017a) it is questionable whether micro and macronutrients should be classified as biostimulants. E.g. N, P, K are primary, Ca, Mg, S are secondary and Fe are micro nutrients. (Hernández-Herrera et al. 2014a) state that the presence of inorganic minerals in liquid seaweed extracts makes them excellent organic fertilizers. According to (Spinelli et al. 2010) kahydrin (vitamin K1 derivative) acidifies the rhizosphere.

1.7 Lipid based molecules

Sterols are an essential group of lipids for eukaryotic plants (Khan et al. 2009). They have listed 24 sterol components for Chlorophyceae (green seaweeds), 14 for Rhodophyceae (red seaweeds) and 11 for Phaeophyceae (brown seaweeds). In Appendix I a table with researches on these components in seaweeds is shown (Type 5). (Hamed et al. 2018) names myristic, palmitic, oleic and eicosapentaenoic acids as examples of abundantly present fatty acids from brown algae. They describe antibacterial activity of these fatty acids from seaweeds against plant pathogenic bacteria. (Ibraheem et al. 2017) for example found that the methanolic extract of *Padina gymnospora* containing a high concentration of palmitic acid showed antibacterial activity against the soil-borne pathogenic bacteria *Ralstonia solanacearum* and *P. carotovora*.

1.8 Secondary metabolites

Secondary metabolites are components such as polyphenols and terpenoids which often have defense or signaling functions (Pereira and Costa-Lotufo 2012). In Appendix I a table with researches on these components in seaweeds is shown (Type 6). (Michalak et al. 2016b) mentions that particularly in brown seaweeds polyphenol concentrations are high. (Chojnacka et al. 2012) mention research that found phlorotannins to have strong antimicrobial activities. (Pereira and Costa-Lotufo 2012) mention antifouling activities of secondary seaweed metabolites.

2 Review of results with seaweed products on crop level

For different crops the tables below give an overview of results after seaweed (product) application. For the setup of the experiments and the detailed results, one should consult the original publications.

2.1 Effects in potato

(Caradonia et al. 2021) wrote a review on the results for seaweed (and other biostimulants) applications on potatoes. Several in vitro, growth chamber, glasshouse and field experiments were described. Seaweed extracts can reduce the required dose of fertiliser without reducing yield. Seaweeds could be considered effective on potato productivity. However, the effectiveness of treatments can vary due to many factors, such as product origin, production process, environmental factors, agricultural practices, timing, weather conditions.

Table 3 *Overview of seaweed applications in potato.*

Author	Crop type	Seaweed (product)	Effect
(Kowalski et al. 1999)	Potato cv. 'BPI'	Kelpak	The addition of 0.25% seaweed concentrate to the medium improved plantlet quality and led to better establishment in the greenhouse. No beneficial effect of seaweed concentrate in the tissue culture medium was observed if a second cutting was part of the micropropagation process.
(Uppal et al. 2008)	Russet Burbank, moderately susceptible and Kennebec, highly susceptible	Liquid seaweed '*Ascophyllum nodosum* L.' 29% concentrate (Acadian Seaplants Ltd., Nova Scotia, Canada)	Seaweed plant extracts showed some efficacy in reducing *Verticillium* wilt severity in growth room trials, but were less effective in the field
(Pramanick et al. 2017)	Kufri-Jyoti variety	Seaweed sap derived from the marine alga K*appaphycus alvarezii*	Results suggested that K-sap with the concentration of 7.5% along with 100% of the fertilizer is the best to improve growth, yield and quality of potato, and this treatment was followed by 5% K-sap + 100% fertilizer. It was also exhibited that 7.5% K-sap has the potentiality to substitute 25% of the fertilizer
(Reis et al. 2018)	Potato-Dextrose-Agar medium	Extracts and dried, milled flour of *Ulva fasciata*	Neither the flour nor ulva extract showed any direct anti-fungal activity, but the presence of compounds produced by *U. fasciata* showing antagonist physiological effects against *S. solani* should be investigated.
(Wadas and Dziugieł 2020)	Potato cultivars ('Denar', 'Lord', 'Miłek')	Seaweed extracts Bio algeen S90 (*Ascophyllum nodosum*) and Kelpak SL (*Ecklonia maxima*)	The biostimulants did not affect dry matter, protein, total sugars, monosaccharides and sucrose or L-ascorbic acid content in new potatoes. Bio-algeen S90 increased the starch content in tubers of all potato cultivars tested, whereas Kelpak SL and HumiPlant reduced nitrates content only in tubers of Denar' cultivar and increased ascorbate-nitrate

			index. The biostimulants did not affect potato after-cooking darkening. Both the nutritional value of new potatoes and after-cooking darkening depended on the cultivar and weather conditions during the potato growing period to a great extent. Conclusions: Plant biostimulants slightly affected quality of new potatoes
(Dziugieł and Wadas 2020)	Potato cultivars ('Denar', 'Lord', 'Miłek')	Seaweed extracts Bio-algeen S90 (*Ascophyllum nodosum*) and Kelpak SL (*Ecklonia maxima*)	The use of biostimulants increased potassium (K) content in tubers. Bio-algeen S90 did not affect the phosphorus (P) content in tubers, whereas Kelpak SL and HumiPlant reduced the phosphorus content. The biostimulants did not affect calcium (Ca), magnesium (Mg), or sodium (Na) content in tubers. The use of biostimulants resulted in an increase in the mass ratios of K^{+}:Ca^{2+}, K^{+}:Mg^{2+}, and (K^{+} + Na^{+}):(Ca^{2+} + Mg^{2+}) in early crop potato tubers, but did not affect the mass ratios of Na^{+}:Ca^{2+} and Na^{+}:Mg^{2+} or the mass ratio of Ca:P. The macronutrient content in early crop potato tubers and their ionic ratios depended on the cultivar and environment conditions.
(Garai et al. 2021)	cv. Kufri Jyoti	Different seaweed extracts, i.e., *Kappaphycus alvarezii* sap (K sap) and *Gracilaria edulis* sap (G sap)	Foliar feeding with 10% K sap along with recommended dose of fertilizer brought about significant enhancement in plant height, being statistically similar with 10% G sap. Similar treatment resulted in a maximum tuber bulking rate and tuber yield accounting for 32.11% and 24.87% yield enhancement over control. Maximum nutrient (N, P, and K) uptake as well as best values of quality traits in terms of ascorbic acid, reducing sugar content, and specific weight of potato tuber were recorded with economically viable treatment having 10% K sap spray.
(Hamed et al. 2018)			Brown algal extracts have been shown to increase the productivity of potato. Furthermore, alginates (specific ingredient polysaccharides in brown algae) have been found to inhibit potato virus X (PVX).
(Craigie 2011)			Cytokinin-like bioactivity was reported in the early 1970s in commercial seaweed extracts and experimental trials with these extracts resulted in increased potato yields
(Asad 2012)	cv. „Sante"	Seaweed extract Primo	A significant improvement in growth, yield and tuber quality of potato was observed where treatment was applied. The treatment also improved nitrogen, total soluble solids and protein contents of the potato tubers

2.2 Effects in onion

Table 4 *Overview of seaweed applications in onion.*

Author	Crop type	Seaweed (product)	Effect
(Abbas et al. 2020)	Four onion cultivars, 'Lambada', 'Red Bone', 'Nasarpuri', and 'Phulkara'	SWE Wokozim, Ascophyllum nodosum extract characterized as a mixture of cytokinins, auxins, and betaines	0.5% SWE increased the yield, nutrient contents, and total soluble solids (TSS) of the four onion cultivars whereas 3% SWE, the highest concentration, increased ascorbic acid in different onion cultivars
(Lola-Luz et al. 2014)	Onion seeds (cv Hybing F1	Cold process seaweed extract Algae GreenTM: Dry seaweed (by-product of the seaweed extract) (OGT, Kilcar, Co. Donegal, Ireland) and seaweed spray	Results from this study indicated that there was an increase in phenolic and flavonoid content in onion. There were no statistically significant differences in yield
(Szczepanek et al. 2017)		Seaweed biostimulant Kelpak SL, extracted from Ecklonia maxima	The biostimulant applied from the three-leaf stage increased the chlorophyll index after double or triple application, whereas applied from the four-leaf stage, also after a single application. The highest increases in the fresh weight yield of bulbs as well as fresh weight of roots resulted from the triple application of the biostimulant from the three- or four-leaf stages. Each dm3 of the biostimulant caused an increase in the fresh weight yield of bulbs by 0.76 t ha-1, and each additional application resulted in an increase in yield by 1.76 t ha-1.
(Gupta et al. 2021)		Seaweed extract Kelpak®	Seaweed treated plants showed the best growth response and had the highest chlorophyll content, compared to plant growth-promoting rhizobacteria. All biostimulant treatments increased the endogenous cytokinin and auxin content. These results suggest that co-application of different biostimulant classes with different modes of action could further increase crop productivity with an improvement in both growth and nutrition content being achieved in onion with the co-application of a seaweed extract and PGPR.
(Dogra and Mandradia 2014)		A. nodosum	Increased in yield and, reduced severity of downy mildew
Bettoni et al, 2010		Seaweed extract	Increased fresh and dry weight of bulbs and decreased loss of bulb biomass during storage

2.3 Effects in corn/maize

Table 5 Overview of seaweed applications in corn/maize.

Author	Crop type	Seaweed (product)	Effect
(Mondal et al. 2014)	*Zea mays*	Pristine j-sap; GA3-free j-sap; IAA-free j-sap and autoclaved j-sap of red seaweed, *Kappaphycus alvarezii*	The vegetative biomass increased dramatically. Heightened photosynthetic activity and corn stover yield.
(Possinger and Amador 2016)	Sweet corn (*Zea mays* L.)	Brown and red seaweed species	Soil electrical conductivity, potassium (K^+), sulfate (SO_4^{2-}), and active carbon (C) increased with seaweed addition relative to the organic fertilizer, whereas potentially mineralizable N and pH decreased, with effects varying over time. Sweet corn yield and quality were either equivalent to that with the organic fertilizer or improved. Negative effects were increased salt levels in the soil
(Navasero et al. 2016)	Corn (*Zea mays* L.)	Brown seaweed, *Sargassum cinctum* J. Agardh	Repellent reaction of the neonates and second instar larvae of *O. furnacalis* to the volatiles from detached leaves of *S. cinctum*
(Jeannin et al. 1991b)	Maize (*Zea mays* L. cv DEA)	Goemar GA 14	Increased the total fresh matter production of maize seedlings by 15 to 25% over the control. This was reflected in the increase of root and stem mass per plant.
(Trivedi et al. 2018)	Maize	*Kappaphycus alvarezii* seaweed extract	Increase of mainly 15% of the seed yield (g/plant) in water optimal conditions through the enhancement of yield parameters as the number of seeds per cob and the cob length
(Singh et al. 2016)	*Zea mays*	Sap from two seaweeds *Kappaphycus alvarezii* (K-sap) and *Gracilaria edulis* (G-sap)	Enhanced the grain productivity. Significant increases in P (35.5 %) and K (14.4 %) content in grains was observed through G-sap application.
(Bradáčová et al. 2016)	Maize (v. *Colisee*)	Algafect, a commercial seaweed extract based on *Ascophyllum nodosum*, *Fucus* spp. and *Laminaria* spp.	Reduced leaf necrosis and enhanced root length density of maize plants subjected to low root zone temperatures
(Ertani et al. 2018)	Maize	Six commercial seaweed extracts from *Laminaria* spp and *Ascophyllum nodosum*	Ability of plants to absorb Ca, Mg, S, Fe, Cu, Mn, Mo, Zn, and B was enhanced significantly in comparison with the control
(Basavaraja et al. 2018)	Maize	*Kappaphycus alvarezii, Gracilaria edulis,* liquid filtrate from fresh seaweed	Enhanced N, P and K uptake (grain + stover) for both extracts
(Rengasamy et al. 2015)	*Zea mays* cv. Border King	Eckol, a phenolic compound isolated from the seaweed *Ecklonia maxima*	Eckol treatment enhanced both growth and biochemical physiology of the maize cultivar used, possibly through synergistic effects with other plant growth hormones
(De Waele et al. 1988)	Maize	Seaweed concentrate prepared from *Ecklonia maxima*, (Osbeck)	An in vitro experiment in which excised maize roots were treated with seaweed extract showed reduction in the reproduction of the nematode *Pratylenchus zeae* by 47–63%.

			However, in a pot experiment, the reproduction of *P. zeae* was not influenced by seaweed extracts
(Blunden et al. 1996)	Maize	Aqueous alkaline extract of *Ascophyllum nodosum*	Higher concentrations of chlorophyll in the leaves of treated plants in comparison to control plants treated with an equivalent volume of water

2.4 Effects in wheat

Table 6 Overview of seaweed applications in wheat.

Author	Crop type	Seaweed (product)	Effect
(de Borba et al. 2021)	Wheat (*Triticum aestivum* L.)	Ulvan, a water-soluble polysaccharide from the green seaweed *Ulva fasciata*	Their findings provide evidence that ulvan confers protection and triggers defense mechanisms in wheat against *Z. tritici* without major modification of the plant physiology
(Zou et al. 2021)	Wheat (*Triticum aestivum*; L. Jimai 22)	A fuciodan from *Macrocystis pyrifera*	The results indicated that MPF could improve the salt tolerance of wheat seedlings
(Zuo et al. 2021)	Wheat (*Triticum aestivum*)	Low molecular weight polysaccharides (LPU) derived from *Ulva prolifera*	Their findings indicate that LPU might have the effect of regulating the abscisic aciddependent pathway in wheat, thereby increasing seedling antioxidant capacity and growth. Application of LPU may accordingly represent an effective approach for enhancing the resistance to osmotic stress in wheat
(Zou et al. 2019)	Wheat (*Triticum aestivum* L. Jimai 22)	Polysaccharides from brown seaweed *Lessonia nigrescens* polysaccharides (LNP)	The results showed that LNP promoted the growth of plants, decreased membrane lipid peroxidation,increased the chlorophyll content, improved antioxidant activities, and coordinated the efflux and compartmentation of intracellular ion. All three polysaccharides could induce plant resistance to salt stress.
(Zou et al. 2018)	Wheat (*Triticum aestivum* L. Jimai 22)	Polysaccharides from *P. yezoensis* (PP)	The results showed that exogenous PP increased wheat seedling shoot and root lengths, and fresh and dry weights, alleviated membrane lipid peroxidation, increased the chlorophyll content and enhanced antioxidant activities. The results demonstrated that polysaccharides could regulate antioxidant enzyme activities and modulate intracellular ion concentration, thereby to protect plants from salt stress damage. Furthermore, there was a significant correlation between the tolerance of wheat seedlings to salt stress and MW of polysaccharides.
(Stamatiadis et al. 2021)	Winter wheat	*Ascophyllum nodosum* extract	Application at the tillering stage increased average yield, grain nutrient accumulation (N, P, K) and N-use efficiency over the three site-year period, but extract effects were not consistent between site-years both in terms of optimal growth stages of application and magnitude of crop responses.
(Vafa et al. 2021)	Wheat cultivars,	Seaweed extract	Application of combination of *Phosphobacteria* sp. + *Azotobacter* sp. and *Azospirillum* sp., mycorrhizal fungus

	namely Sardari and Sirvan Sanandaj		and seaweed extract improves growth parameters and grain yield in wheat.
(Stamatiadis et al. 2015)	Winter wheat	*Ascophyllum nodosum* seaweed extract (AZAL5)	AZAL5 application caused increased grain K uptake and an increase in yield only when mineral N was added. Differences in the efficacy of the two AZAL5 concentrations indicated that optimal dilution ratios were directly or indirectly dependent on soil water content.
(Laurent et al. 2020)	Durum wheat var. Miradoux	DPI4913 containing *Ascophyllum nodosum* extract	The extract improved yield (grain biomass), and N recovery in whole plants at maturity was enhanced.
(Nasiroleslami et al. 2021)	Wheat (*Triticum aestivum* L.) (cv. SHS 022)	Seaweed extract	The results showed 150 kg N ha^{-1} along with humic acid and seaweed extract have the greatest effect on wheat yield. The high amount of N increased palmitic acid but decreased linolenic acid
(Latique et al. 2021)	Wheat plants (*Triticum durum* L., variety Karim)	Sprays obtained from *Ulva rigida*	The obtained results indicated that seaweed treated plants showed higher ability to tolerate salt stress by a significant increase of plant growth and the photosynthetic pigment contents, compared to those of control (non-treated plants). Furthermore, there was a significant improvement in antioxidant enzyme activity, such as superoxide dismutase (SOD), isocitrate dehydrogenase (ICDH), glutathione peroxidase (GPx), glutathione reductase (GR) activities in the stressed plants.
(Latique et al. 2017)	Durum wheat	Liquid SWEs made from brown seaweed, *Fucus spiralis*	Application of SWE at different concentrations significantly enhanced seed germination and growth parameters under salt stress. Results show that the activity of antioxidant enzymes increased with increasing the algal extract concentration.
(Gunupuru et al. 2019)	Wheat (*Triticum aestivum*) cultivar 'Helios'	*Ascophyllum nodosum* liquid SWE (LSE)	Systemic disease resistance appears to be induced by LSE and chitosan in response to *F. graminearum* in wheat by inducing defense genes and enzymes.
(Michalak et al. 2016a)	Winter wheat (variety Akteur)	*Ascophyllum nodosum* and Baltic green macroalgae	Formulations containing supercritical algal extracts showed similar biostimulant properties as products available on the market.
(Pačuta et al. 2021)	Durum wheat (*Triticum durum* Desf.)	Biofertilizers Alga 300++P and Alga 300++K based on brown seaweed extract	Foliar application of bioactive substances led to a significant increase in the yield of durum wheat while maintaining or increasing the quality parameters of the grain.
(Salim 2016)	Wheat (*Triticum aestivum* L.) cultivar Sakha 93	Seaweed extract UAD Company	Obtained results revealed that, adding biochar, sprayed seaweed extract treatments individually or in combination have stimulating effect on the most of morphological characters and yield components as compared with control plants in two seasons.
(Paulert et al. 2010)	Wheat (*Triticum aestivum* cv. Prelude-Sr5) cell-suspension	Ulvans from green seaweed *U. fasciata*	Pretreatment of whole plants with ulvan significantly reduced the symptom severity of *Blumeria graminis* infection, by 45% in wheat. Thus, the priming activity of ulvan on the oxidative burst correlates with a decrease of disease symptoms in infected plants.

	cultures and wheat (cv. Kanzler)		
(Beckett and van Staden 1989)	Spring wheat (*Triticum aestivum* L., cv. SST 66)	Kelpak	Kelpak had no significant effect on the yield of wheat receiving an adequate K supply, but significantly increased the yield of K stressed plants. The increase in yield was caused by an increase in both grain number and individual grain weight.
(Sharma et al. 2019)	Wheat variety, Sharbati Tukdi	*Gracilaria dura* (GD) sap	GD-sap application conferred drought tolerance (as the biomass increased by up to 57% and crop yield by 70%), via facilitating physiological changes associated to maintaining higher water content. GD-sap application significantly increased ABA accumulation due to enhanced expression of biosynthesis genes. Moreover, GD-sap application enhanced the expression of stress-protective genes specifically under water stress.
(Shahbazi et al. 2015)	Wheat var. Chamran	Seaweed liquid fertilizer (SLF) of *Ulva fasciata*, *Nizimuddinia zunardini* and *Gracilaria corticata*	The seeds soaked with aqueous extract of seaweeds performed better when compared to the water soaked controls
(Carvalho et al. 2014)	'IAC 364' wheat	*Ascophyllum nodosum* extract	Plants irrigated with *A. nodosum* extract showed increments in the height, dry mass of shoots and number of spikes, however these plants had the lowest harvest index when compared to the control. Seed treatment also increased plant height, but it did not change biochemical and productivity parameters
(Zodape et al. 2009)	Wheat (*Triticum aestivum* L.)	*Kappaphycus alvarezii* extract	Compared to control the yield of grain increased. The nutritional quality of grain such as carbohydrate, protein and minerals also improved under the influence of treatment
(Sen et al. 2015)	Wheat (var. HUW 468)	Liquid formulation sprays of a seaweed extract from *Ascophyllum nodosum* commercially known as Biovita	At some concentrations, the performance of wheat was improved, as well as grain and straw yields and protein content
(Ibrahim et al. 2014)	Wheat (*Triticum aestivum* L.)	Water extract of *Ulva lactuca*	Algal presoaking of grains demonstrated a highly significant enhancement in the percentage of seed germination and growth parameters. The activity of superoxide dismutase (SOD) and catalase (CAT) increased with increasing the algal extract concentration while activity of ascorbate peroxidase (APX) and glutathione reductase (GR) was decreased with increasing concentration of algal extract more than 1%(w/v). The protein pattern of wheat seedling showed 12 newly formed bands as result of algal extract treatments compared with control
(Nelson and Staden 1986)	*Triticum aestivum* L. cv. Inia	Kelpak 66, from *Ecklonia maxima*	Production of root and shoot dry mass and kernel mass increased
(Kasim et al. 2015)	*Triticum aestivum*	Seaweed extracts (*Sargassum latifolium*, *Ulva lactuca*)	Pretreatment with seaweed extract of *Sargassum* or *Ulva* led to the alleviation of damaging effects of drought on *Triticum aestivum* during vegetative stage while a mix of

			the two types of seaweed extracts resulted in antagonistic effect
(Shah et al. 2013)	Wheat var. 'GW 496'	*Kappaphycus alvarezii* and *Gracilaria edulis* sap	It was found that yield of grain was increased significantly over control. The increase in yield was attributed to increases in the number of spike, spike weight, spike length and 100 seed weight. Some nutrient contents in the grains were increased

2.5 Effect on soil and plant nutrition

As stated in table 2 seaweed (extracts) can be used as bio-stimulant. However, the seaweed itself can be used as fertilizer, or the so called green manure. It can be used as an improvement of the soil conditions/health and at the same time it can increase the nutritional values of the soil for the plants. For soil health holds that the most effect is seen at clay soils with low organic matter, these soils are not porous. Adding seaweeds adds humic acid and alginates creates a more crumby structure. (Zodape 2001). Besides the salts of alginic acid form high-molecular-weight complexes with the metallic ions in the soil, These complexes absorb moisture, swell and retains soil moisture improving the soil aeration and capillary activity of the soil pores, stimulating plant-root growth (Khan et al., 2009). Adding seaweed to the soil has in this case the same effect as increasing the organic matter content using regular compost. To increase the OM% of a soil with 1% there is a approximately 500 tons of compost / seaweed needed. However, not adding any OM to the soil will lower the OM% further.
Regarding the nutritional values the macro- (N,P,K) and micronutrients of the seaweed are of main importance. The nutritional values between seaweed spices differ a lot, regarding Raghunandan (et al., 2019). In Table 7 the macro nutrients of some seaweed extracts can be found, in Table 8 the micro nutrients.

Table 7 macro nutrient content different seaweed species, taken from Raghunandan et al. 2019

Name of seaweed	**Type**	**Nitrogen (mg/g)**	**Phosphorus (mg/g)**	**Potassium (mg/g)**	**References**
Sargassum wightii	Brown algae	174.02	45.56	72.83	Divya et al. (2015a)
Dictyota dichotoma	Brown algae	175.02	44.56	71.84	Sasikumar et al. (2011)
Laurencia obtuse	Red algae	3.9	3.8	2.0	Safinaz and Ragaa et al.
Corallina elongate	Red algae	3.4	3.8	1.6	(2013)
Jania rubens	Red algae	4.0	3.5	1.6	
Ulva lactuca	Green algae	174.02	45.56	75.83	Divya et al. (2015b)

Table 8 micro nutrient content different seaweed species, taken from Raghunandan et al. 2019

Mineral compounds (ug/g of extract)	**Red algae (*Lithothamnion calcareum*)**	**Green algae (*Ulva lactuca*)**	**Brown algae (*Stoechospermum marginatum*)**
Copper	4.89	0.38	8.64
Manganese	57.50	62.00	8.75
Zinc	15.80	1.01	19.92
Iron	915.00	0.37	858.50
Potassium	5.17	113.00	29.65

Magnesium	25.80	18.30	9.60
Cobalt	0.08	0.06	3.47
Chromium	0.82	Nd	16.60
Lead	0.15	Nd	0.40
Nickel	1.84	10.40	25.20
Cadmium	0.07	2.00	5.90
Sodium	4.15	185.00	39.11
Calcium	351.50	195.26	2053.40
Source	Aslam et al. 2010	Aslam et al. 2010	Aslam et al. 2010

Besides extracts, the seaweed can be used as a fertilizer in a mulched, powdered or composted way. Doing this the seaweed is used as a whole, including all the cell walls. Regarding using whole seaweed as fertilizer it has to follow regulations. According to Dutch law (uitvoeringsbesluit meststoffenwet) only small amounts of heavy metals / micro nutrients are allowed within compost (see Table 9).

Table 9 maximum allowed quantity of heavy metals in compost (Dutch "Uitvoeringsbesluit meststoffenwet")

Component	Maximum allowed quantity (mg per kg dm)
Cadmium	1
Chromium	50
Copper	90
Mercury	0.3
Nickel	20
Lead	100
Zinc	290
Arsenicum	15

Furthermore, the NPK content of the (dried) seaweed is of importance for the usability as a fertilizer. There are many sorts of seaweed, in Table 10 the heavy metal and NPK content of some eadible seaweeds are shown. The NPK content of the green seaweed (Clorophyta) is 1:0.04:0.15, of the brown seaweed (Phaeophyta) 1:0.03:0.75 and for the red seaweed (Rhodophyta) 1:0.03:0.01. As can be seen in Table 10 the Rhodophyta *Acanthopeltis japonicus* exceeds the maximum zinc content. All the other seaweeds do not exceed the heavy metal concentrations.

Table 10 NPK and mineral content of different seaweed species (mg/g DW)

taxon	species	N	P	K	Na	Ca	Mg	Si	Sr	Fe	Al	Zn	B
Clorophyta	*Monostroma nitidum*		0,9	7,6	17,9	13,6	13,6		0,18	0,9	1,15	0,21	0,05
Clorophyta	*Ulva pertusa*	33	1,45	5,1	3,6	8	25,8	5,2	0,22	0,76	0,56	0,14	0,065
Clorophyta	*Ulva conglobata*		0,8	1,7	1	8,3	36,5			0,61	1,01	0,06	
Clorophyta	*Enteromorpha compressa*					11,9	19,8	21,8	0,33	1,13	0,69	0,25	0,116
Clorophyta	*Chaetomorpha crassa*		0,8	13,2	6,5	10,3	11,3		0,23	0,36	0,56	0,15	0,14
Phaeophyta	*Padina arborescens*		0,98	35,7	16,1	19,2	8		1,31	0,84	0,77	0,14	0,13
Phaeophyta	*Ishige foliacea*					12,4	9,1	13,9	1,17	0,41	0,14	0,17	0,073
Phaeophyta	*Scytosiphon lomentaria*		1,8	8,7	9,9	31,9	10,7	2,5		1,49	1,4	0,19	0,053
Phaeophyta	*Eisenia bicyclis*	29,6	0,9	22,1	14,8	15	9		1,1	0,1	0,1	0,1	0,097
Phaeophyta	*Hizikia fusiforme*		0,9	34,3	16,9	17,2	9,9		1,14	0,16	0,2	0,08	0,109
Phaeophyta	*Sargassum ringgoldianum*		0,75	17,4	2,2	20,8	10,7		1,48	0,11	0,1	0,06	0,097
Phaeophyta	*Sargassum tortile*		0,7	5,4	2,7	28,8	8,7		1,98	0,11	0,08	0,17	0,066
Phaeophyta	*Sargassum thunbergii*		1,1	20,9	8,8	26,2	9,7	68	1,65	0,71	1,03	**0,32**	0,122
Rhodophyta	*Gelidium amansii*	28,9	0,9	0,3	0,2	7	5,3	3,3	0,09	0,36	0,14	0,16	0,177
Rhodophyta	*Acanthopeltis japonicus*		1,05	1,8	1,8	1,1	4,8		0,02	0,23	0,32	0,17	
Rhodophyta	*Carpopeltis flabellata*		1,2	6,5	10,7	3,8	7,8			0,3	0,24	0,17	0,055
Rhodophyta	*Gloiopeltis tenax*					5,1	3,3		0,03	0,16		0,08	0,034
Rhodophyta	*Gymnogongrus flabelliformis*					2,8	4,2		0,09	0,28		0,12	0,217
Rhodophyta	*Chondrus ocellatus*					8,3	10,8			0,43		0,17	0,076
	Source:	Ochiai et al., 1987	Yamamoto et al., 1979										

For *Sargassum* species (taxus Phaeophyta) in the Caribbean and Florida the heavy metal concentrations and NK content was determined by Lopez-Contreras et al., 2021, see Table 11. Sargassum is in the Caribbean a problem, this seaweed is threatening endangered coastal ecosystems, like coral reefs, besides flooding the beaches. Using this seaweed as fertilizer could be a solution. However, all harvested *Sargassum* samples have to high in-organic and total Arsenic concentrations, besides two of them have also to high cadmium concentrations (bolt in Table 11). The too high cadmium concentrations where also observed in *Ulva clathrata* grown in tanks (Pena-Rodriguez et al., 2011).

Table 11 Element concentrations in mg/kg DW in *Sargassum* from the Caribbean and Florida López-Contreras et al., 2021

Country	Harvest place	Cd	Hg	Pb	tAs	iAs	I	N	K
Bonaire	Lagun	**1,5**	<0,020	8,4	**89**	**56**	221	12000	60800
Bonaire	Lac Bay	**1,2**	<0,022	0,5	**74**	**44**	403	12000	62900
USA	Florida Coast	7	<0,018	0,7	**76**	**48**	106		
St. Maarten	open sea	0,4	<0,023	3,4	**111**	**89**	120	800	
St. Maarten	open sea	0,3	<0,026	7,3	**133**	**99**	111	900	
St. Maarten	Point Blanche bay	0,6	<0,087	1	**42**	**31**	139	700	
St. Maarten	Guana bay	0,4	<0,036	0,6	**36**	**18**	140	1300	
Mexico	Cancun	0,5	<0,017	0,3	**115**	**77**	140		

Within the *Sargassum* species there is also a difference in NPK content, dried *Sargassum wightii* has a NPK ratio of 1:0.1:1.8 (Kaladharan et al., 2021), while Lopez-Contreras (et al., 2021) found an N:K of 1:~5. Which both is completely different from the values in Table 10. Concluding from this the statement: "Seaweed can be used as a fertilizer" is dependent on the species, where heavy metal content should be taken into account. Meaning some species will be usable, some not.

3 Experiments in the Netherlands with Seamel Pure from Olmix

3.1 Onion field experiments

Introduction

In 2018 and 2019, a test on various potassium fertilisation strategies and plant stimulants was carried out at the Wageningen Research test farm in Lelystad. The treatments involved varying the dose and application pattern (single, split-dose) of the plant stimulant and method of application (via the soil or as a foliar treatment). An additional treatment was included to test the effects of the seaweed extract of Olmix for 2018 and 2019. This was sprayed over the plants. The effect on the resilience of the plants and quality of the onion bulbs was evaluated. A few rows of onions per treatment were sown at such a high density that plant diseases were provoked. The research is described in detail in Van Geel et al., 2019 en 2020, the reports are in Dutch and anonymised (Appendix 2). In the paragraph below a summary of this research is given. The for seaweed extract relevant treatments are shown in *Table 12*.

Table 12 *Objects seaweed extract product.*

Object 2018	Object 2019	Description	
A	AB	Reference untreated	Fertilizer NPK
Q	F	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha just before bulbing and two weeks later second treatment
R	G	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha just before bulbing and two weeks later second treatment
S	H	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha in the 3-leave stage and just before bulbing

Results

The plant density in 2018 was somewhat low with on average 76 plants per m² (goal is 90 plants per m²). The drought during the summer of 2018 can be the explanation for this, although because of irrigation of the crop the yield was not considered bad (40 ton/ha). Size of the onions was 35-60 mm which is rather small. No significant differences were observed during the growing season of 2018 between the different treatments (crop status, crop regularity, colour of the crop and foliage falling and dying) (see *Table 13*). During the dying process of the leaves Stemphylium and Fusarium were present. But no significant differences in damage between the treatments were observed.
Because no significant positive or negative effects on growth and quality were observed in 2018 also no significant higher or lower yields and market value were registered (*Table 14*).

In 2019 on average 81 plants per m^2 were present. Only in object C a significantly lower plant density was determined (75 plants per m^2). During growing season no other significant differences in crop condition between the objects was observed. There was a increase in yield after applying 2 l/ha of Seamel at 3 leaves and before bulbing, but this was not significant. Leaf diseases were low as was also the case in 2018, this was probably due to the dry summers. The crop yield was higher than in 2018 (63 ton/ha). No significant differences were observed during storage and market value between the different objects. The harvested amount of bulbs in 2019 was higher than in 2018 but storage of the bulbs did have more effect on the hardness of the bulbs in 2019. The hardness of the bulbs after applying seaweed extracts seemed to be slightly higher but wasnot significant. Only the decrease in weight was significantly lower but only 2% after applying seaweed extract.

Table 13 *Observation crop status 2018 and 2019.*

Datum	Crop status	Crop regularity	Colour crop	Falling of the leaves	Percentage green leaf
2018					
15 June	7,5	8,0			
29 June	6,3	7,3			
13 July	6,8	7,8	7,9		
27 July	7,2	7,8	8,8		
13 Aug				75%	74%
24 Aug				94%	50%
31 Aug				97%	25%
2019					
1 juli	7				
15 juli	8,8				
22 juli	9,0				
29 juli					
5 aug				30%	90%
12 aug				95%	90%
26 aug					48%
2 sep					23%

Table 14 *Yield, after harvest, storage efficiency, and market value 2018.*

Object	Description	Yield (ton/ha)		Market	Storage efficiency
2018		**Fresh**	**Dry matter**	**(ton/ha)**	
A	Reference	47,4	7,7	38,5	81%
Q	Seamel Pure 1	47,2	7,0	38,7	82%
R	Seamel Pure 2	46,0	7,2	35,3	77%
S	Seamel Pure 3	48,8	8,0	39,9	82%
F pr.		*n.s.*	*n.s.*	*n.s.*	*n.s.*
Object	**Description**	**Yield (ton/ha)**		**Market**	**Storage efficiency**
2019		**Fresh**	**Dry matter**	**(ton/ha)**	
AB	Reference	76,9	11,3	64,1	83%
E	Seamel Pure 1	75,3	11,0	62,6	83%
F	Seamel Pure 2	76,4	10,6	63,6	83%
G	Seamel Pure 3	78,6	11,1	66,1	84%
F pr.		*n.s.*	*n.s.*	*n.s.*	*n.s.*

Figure 4 *Experimental field onions in 2018.*

Another important parameter that was measured is the hardness of the bulbs. Also for the hardness no significant differences between the treatments were observed (*Table 15*).

Table 15 *Hardness before and after storage (index) 2018.*

Object	Description	Before storage	After storage
2018			
A	Reference	100	88
Q	Seamel Pure 1	99	84
R	Seamel Pure 2	100	80
S	Seamel Pure 3	103	82
F pr.		*n.s.*	*n.s.*
2019			
AB	Reference	99	59
E	Seamel Pure 1	99	62
F	Seamel Pure 2	101	62
G	Seamel Pure 3	101	62
F pr.		*n.s.*	*n.s.*

In *Table 16* the mineral uptake of the union bulb per hectare is shown. There are no significant differences between the uptake of minerals for the different treatments.

Table 16 *Mineral uptake of the onions (kg/ha) 2018.*

Object	Description	N	P_2O_5	K_2O	SO_3	MgO	CaO
2018							
A	Reference	123	54	134	89	11	80
Q	Seamel Pure 1	109	50	127	83	10	80
R	Seamel Pure 2	111	53	123	80	10	82
S	Seamel Pure 3	126	58	134	85	11	83
F pr.		*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
2019							
AB	Reference	161	61	171	98	18	9,7
E	Seamel Pure 1	164	60	168	97	19	10,1
F	Seamel Pure 2	152	59	167	92	17	9,2
G	Seamel Pure 3	158	59	170	98	18	9,7
F pr.		*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Between the different treatments no significant differences were observed. This can partly be explained by the dry summer of 2018 the crop was hardly influenced by diseases (less than 1 % foliar diseases were observed). For 2019 also no significant differences were observed between the different treatments.

The results of both years were statistically analysed. Some significant differences were found but they were mostly related to the other tested product, and these differences were too small or unimportant to be worth consideration.
All statistical analyses have been done with an uncertainty of 5% which is normal procedure for crop protection products. However in parallele to the new EC 1009/2019 harmonized regulation there are some official XP-CEN Technical Specification for biostimulant testing methods and support for biostimulant claims building (ANFAR, 2022). As biostimulants impacts are generally small compared to those of PPP products, the significant threshold value in statistical analysis of trials could be, in place of the classical α=5% for ANOVA + parametric or non-parametric tests:

- 10% for controlled condition trials
- 15% for field trials

If this had been done this probably could change some tendencies. But still the effects in these years were so small (3% markable yield in 2019) that we didnot repeat the statistical analyses.

The weather during the experiment in 2018 was for Dutch conditions not representative for average summers. It was typed as a hot and dry summer, this certainly effected the experiment. The fact that little diseases were registered at the high density rows of the control confirms this. In 2019 the weather was less different from average but still typed as a hot and dry summer.

3.2 Potato field experiments

3.2.1 2019 field experiment potatoes

In 2019 a field experiment was set up with potatoes. This research is reported in an anonymised reports (see Appendix 2) In the paragraphs below a summary of this research is given.

The cultivated potato plants (cv. Agria) were grown at Wageningen University and Research location Lelystad. The experiment was treated conform local good agricultural practice. A plot consisted of 3 meters (4 rows) of 11 meters. The trial was carried out in four replications. Different alternative foliar sprays were compared to the reference (no treatment). A no treatment, BCD spraying of humic acid product, EFG spraying with seaweed extract.
Disease observations were carried out once a week. The number of infected leaves was counted, and percentage infected foliage was calculated or percentage necrotic foliage per plot was estimated.
The Standard Area under Disease Progress Curve (StAUDPC) was calculated (indication for disease development during the growing season).
The crop was harvested. Tubers were sorted out, weighed and counted, before storage. After storage rotten tubers were sorted out weighed and counted. The rest of the potatoes were weighed and counted. The for seaweed relevant treatments are shown in *Table 17*

Table 17 *Objects seaweed extract product.*

Object	Description	
A	Reference untreated	
E	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 0.5 L/ha weekly spraying start at12 June till 21 August
F	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha weekly spraying start at12 June till 21 August
G	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha weekly spraying start at12 June till 21 August

Due to the dry and hot weather in June and July 2019 the late blight epidemic developed moderately. By the end of August the untreated reference reached a disease severity level of almost 100% and disease assessments were stopped. In *Figure* the effect of the different treatments can be seen for late blight development.

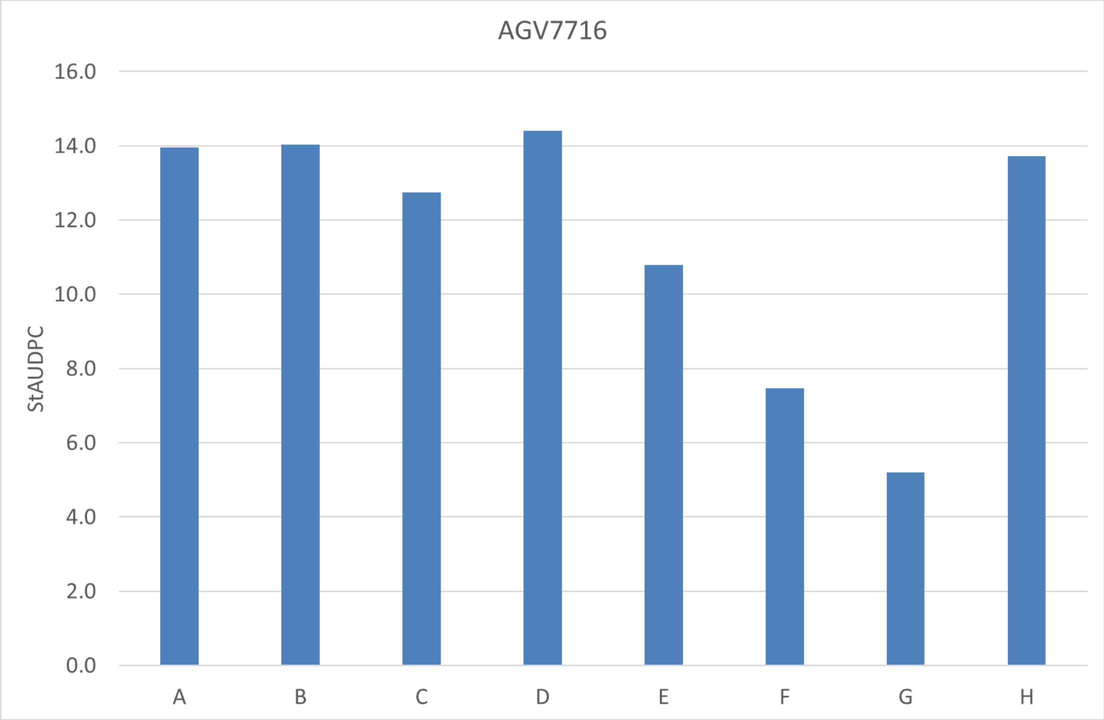

Figure 5 *Potato late blight StAUDPC as a result of various spray schedules.*

Conclusion

- No phytotoxicity was observed, the biological crop protection products used were crop safe.
- Based on the StAUDPC, treatments E, F and G showed a significant efficacy to control potato late blight, where treatment G performed the best, followed by treatment F and in turn followed by treatment E.
- Yield of treatment G was significantly higher than all other treatments. Treatment E and F were similar to the untreated control (A).

Figure 6 *Experimental field potato in 2019.*

3.2.2 2020 field experiment potatoes

The cultivated potato plants (cv. Agria) were grown at Wageningen University and Research location Lelystad. The experiment was treated conform local good agricultural practice. A plot consisted of 3 meters (4 rows) of 11 meters. The trial was carried out in four replications. Different alternative foliar sprays were compared to the reference (no treatment). A no treatment, CDE spraying with seaweed extract and other treatments (F untill N).
Disease observations were carried out once a week. The number of infected leaves was counted, and percentage infected foliage was calculated or percentage necrotic foliage per plot was estimated.
The Standard Area under Disease Progress Curve (StAUDPC) was calculated (indication for disease development during the growing season).
The for seaweed extract relevant treatments can be found in *Table 18*.

Table 18 *Objects seaweed extract products.*

Object	**Description**	
A	Reference untreated	
C	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 0.5 L/ha weekly from 10 Jun to 24 August
D	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha weekly from 10 Jun to 24 August
E	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha weekly from 10 Jun to 24 August
F	JROL extract (Seamel without additional minerals) Foliar spraying:	Foliar 2 L/ha weekly from 10 Jun to 24 August

At the first half of July weather conditions were conductive for potato late blight. The weather conditions in the second half of July and the first half of August were dry. The first part of August was characterised by warm weather on which on several days temperatures were higher than 30°C. In the night of 13 of 14 August about 115 mm water fell followed by another 35 mm in the next four days.

By the end of August the untreated reference reached a disease severity level of almost 90% and disease assessments were stopped. In *Figure* the effect of the different treatments can be seen for late blight development.

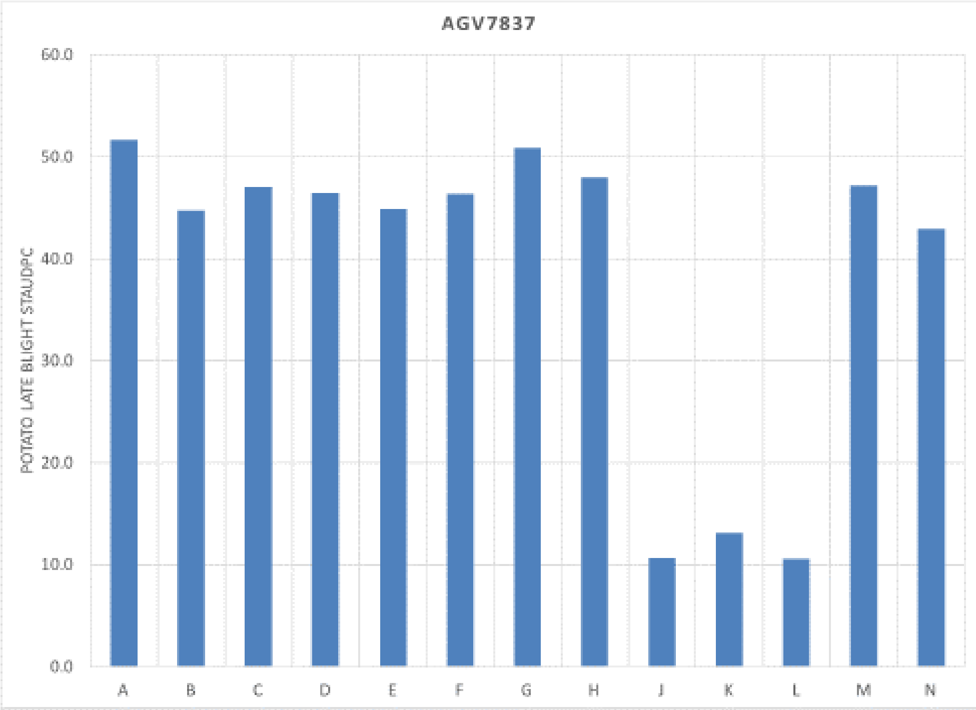

Figure 7 *Potato late blight StAUDPC as a result of various spray schedules.*

The infection period was the first half of July, followed by dry and warm weather. The potato late blight severity was very low until half July.

Conclusion

- No phytotoxicity was observed, the biological crop protection products used were crop safe.
- Based on the StAUDPC the Seamel Pure treatments (C, D, E) showed no efficacy to control potato late blight, disease severity was comparable to the untreated control at the last observation date.

3.2.3 2021 field experiment potatoes

The cultivated potato plants (cv. Agria) were grown at Wageningen University and Research location Lelystad. The experiment was treated conform local good agricultural practice. A plot consisted of 3 meters (4 rows) of 11 meters. The trial was carried out in four replications. Different alternative foliar sprays were compared to the reference (no treatment). A no treatment, B & L sprayed with seaweed extract and other treatments.
Disease observations were carried out once a week. The number of infected leaves was counted, and percentage infected foliage was calculated or percentage necrotic foliage per plot was estimated.
The Standard Area under Disease Progress Curve (StAUDPC) was calculated (indication for disease development during the growing season).
The for seaweed extract relevant treatments can be found in *Table 19*.

Table 19 *Objects seaweed extract product.*

Object	Description	
A	Reference untreated	
B	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha weekly from 28 Jun to 9 August
L	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha weekly from 28 Jun to 9 August

Due to a lot of rain in May the potatoes were planted in the first week of June, which is a month later than planned. In the first half of July weather conditions were conducive for potato late blight which coincided with emergence of the plants. In the second half of July and the first half of August it was in general cool with regular rain showers. Which is less conducive for potato late blight. In *Figure* the effect of the different treatments can be seen for late blight development.

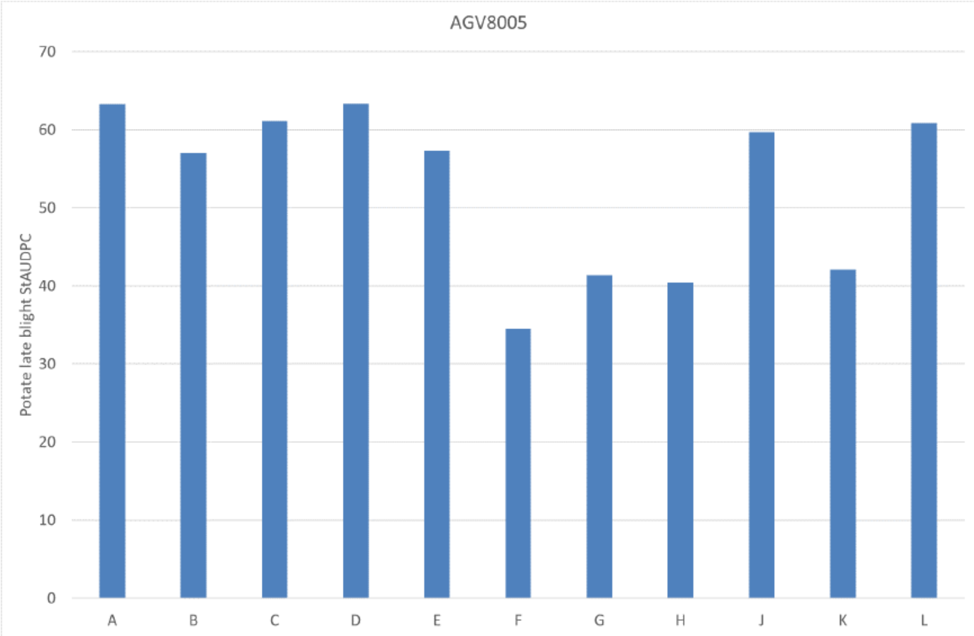

Figure 8 *Potato late blight StAUDPC as a result of various spray schedules.*

Conclusion

- No phytotoxicity was observed, the biological crop protection products used were crop safe.
- Based on the StAUDPC the Seamel Pure treatment (L) and the C, D, J treatment showed no efficacy to control potato late blight, disease severity was comparable to the untreated control.
- Based on the StAUDPC the higher concentration Seamel Pure treatment (B) and treatment E, F, G, H and K significantly controlled potato late blight.
- The efficacy of treatment B to control potato late blight was significantly less than the copper reference (K).

3.2.4 Development of *phythopthora in field experiments during 3 years*

However over the complete season 2020 not significantly, the development of the *Phythoptopthora* in potato after applying Seamel in the different years was slower in all years. The biggest effects were after a late start of the infection as in 2019. The leaves of the potato arenot developing very fast as in the start of the season so probably the coverage of the leaves and/or the time to induce the plants for resistance is best.

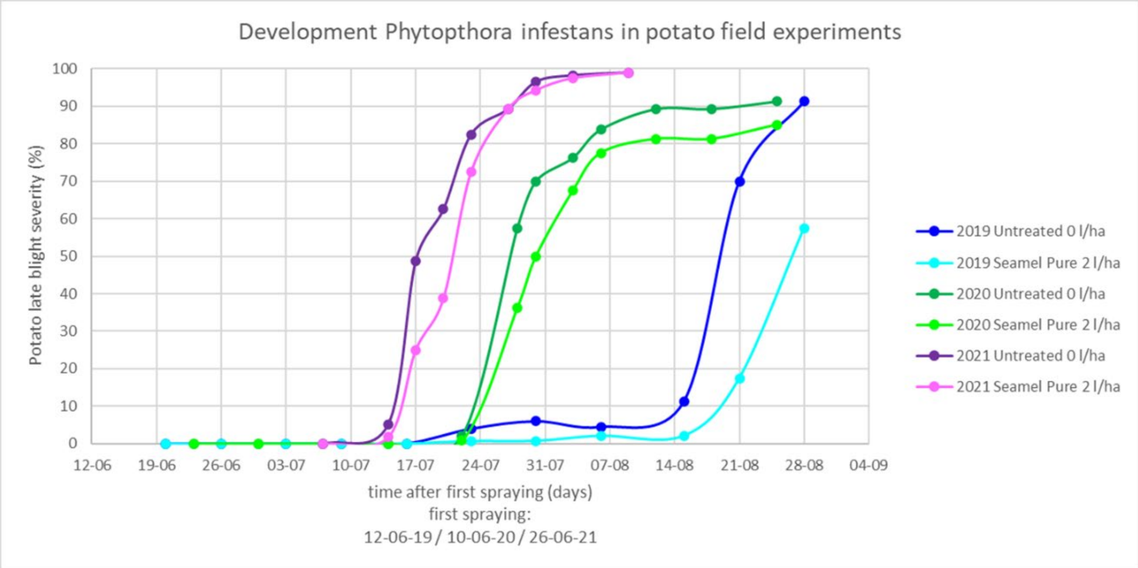

Figure 9 *Development of Phythopthora infestans in field experiments in potato in weekly treated plots with 2 l/ha Seamel or no treatment.*

3.3 Tomato pot experiments

3.3.1 2018 pot experiment tomatoes

A pot experiment with tomato plants (cv. Albis) was carried out to investigate the effect of the Seamel Pure seaweed extract on late blight disease development (More information Appendix 2). It was a first explorative study. The tomato plants were grown in a greenhouse (*Figure*) and were inoculated with late blight. The plants were then sprayed with different doses of humic acid product, seaweed extract and a fungicide. The percentage of necrotic foliage on four leaves per plant was estimated visually.
The main conclusions of the research are:

- No phytotoxicity was observed and the products used were safe for crops
- Late blight severity was significantly lower in all treatments tested than in the untreated control, regardless of the dose rate or the spraying interval
- The fungicide product showed significantly greater efficacy in controlling late blight disease than the other treatments.
- The seaweed extract was significantly better than the humic acid treatment
- Applying the seaweed extract one day before inoculation with a high dose rate was more efficient than a lower dose rate or an earlier application of the high dose rate.

Figure 10 *Experimental set up explorative study late blight with tomato plants.*

Further field experiments with potatoes are recommended, but to achieve the same result as with the fungicide, spraying with alternative products should be complemented with other measures.

3.3.2 2021 pot experiment tomatoes

A pot experiment with tomato plants (cv. HANAMI-cherry) was carried out to investigate the effect of the Seamel Pure seaweed extract on late blight disease development. It was a similar experiment as in 2018, including new treatments and a 66% higher inoculation was used (more information in Appendix 2). The tomato plants were grown in a greenhouse and were inoculated with late blight. The plants were then sprayed with different doses of algae product and seaweed extract or other treatments of which one was 45% copper. The percentage of necrotic foliage on four leaves per plant was estimated visually. The main conclusions of the research are:

- No phytotoxicity was observed and the products used were safe for crops
- Late blight severity was significantly lower using coper or the Seamel Pure seaweed extract treatments tested than in the untreated control, regardless of the dose rate or the spraying interval.
- Applying the seaweed extract both seven days and one day before inoculation with a high dose rate was more efficient than only the day before inoculation and significantly better than the copper treatment. Only the day before inoculation gave a similar efficiency against late blight as the copper treatment. Lowering the dose resulted in less efficiency. The other treatments didn't show any efficiency against late blight.
- The unformulated Seamel object JROLM and the own produced and not formulated *Saccharina latissima* extract were not effective.

Figure 11 *Tomato plants 9 days after inoculation with P. infestans, treatments A, B, C, E, F, G, H and J.*

4 Conclusions field and pot experiments and recommendations

Based on the experiments, it can be concluded that:

- Seaweed extract Seamel Pure of Olmix can be sprayed with normal field sprayers. Crop spraying with doses 0.5-2 l /hectare per spraying seemed safe for onion (2 sprayings), potatoes (8-11 spayings) and tomato plants (2 sprayings). No phytotoxicity was observed.
- In controlled experiments (the pot experiments with tomato) Late blight severity was significantly lower using coper or the Seamel Pure seaweed extract treatments tested than in the untreated control, regardless of the dose rate or the spraying interval. The optimal times spraying with Seamel in the highest dose even gave a significantly better result than the Copper treatment. Lowering the dose of Seamel resulted in less effect. The Olmix seaweed extract without added minerals and the produced Saccharina extract without minerals was not effective.
- Using seaweed extract within onion didn’t show an effect. Both years of onion field experiments were done in a hot and dry summer. This weather resulted in a low disease pressure. If there is any effect should be tested in a season with a higher disease pressure.
- Using seaweed extract within potato’s showed an significant effect based on the StAUDPC (parameter descibing the *Phythopthora* development during the season) and the late blight development with a higher concentration having a bigger effect. However, this is dependent on the weather and the season. When there is a low disease pressure due to the weather, high concentrations still gave the best results. When the decease pressure is high and early in the season due to the weather, neither of the tested concentrations was effective during a longer period.
- An extra repetition of the experiments with a more average weather, including higher concentrations of seaweed extract in different formulations could be wise.
- Marketing of seaweed products requires further proof of products. Further experiments are needed with a better comparison of the effect of formulation and the seaweed composition.

5 Analyses of the seaweed extract

5.1 Metabolomics analysis methods seaweeds and seaweed extracts

5.1.1 Material

The following material has been analysed as shown in Table 12. In total, 11 different seaweed species (freeze-dried material of fresh plants) were used in the metabolomics analysis, plus commercial Seamel batches obtained from Olmix (2018, 2019, 2020, 2021) used at the business unit Field Crops or at Bioscience, a Seamel batch that did not contain the additives that are normally added to the extract (Seamel JROLM), three different extracts of *Ascophyllum nodosum* as prepared by Wageningen Food & Biobased Research (described in W-FBR progress report of "Maatschappelijk Innovatie Programma" AF-16202 Seaweed for food and feed) and as control three other biostimulant products (Vidi fortum, KC2102 and Nordox 45 WG).

Table 15 *Samples used for metabolomics analysis*

Sample
Seamel 2018 (16.04.2018)
Seamel 2019 (21.01.2019)
Seamel 2019 (04.02.2019)
Seamel 2019 (used at field testing by business unit Field Crops; OT)
Seamel 2020 (batch business unit Bioscience)
Seamel 2020 (batch Wageningen-Food and Biobased Research; FBR)
Seamel 2021 (batch business unit Field Crops; OT)
Seamel 2021 JROLM (Olmix batch without additives)
Saccharina latissima, aqueous extract (W-FBR)
A. nodosum, acid extraction (HCl, pH3)
A. nodosum, neutral extraction
A. nodosum, alkali extraction (NaOH, pH9)
Vidi fortum
KC2102 powder
Nordox 45 WG powder
Fucus serratus
Ascophyllum nodosum
Undaria pinnatifida

Laminaria digitata
Saccharina latissima
Chondrus crispus
Palmaria palmata
Gracilaria gracilis
Porphyra umbilicalis
Asparagopsis armata
Ulva lactuca

5.1.2 Methods
Samples were analysed via LCMS (liquid chromatography coupled to mass spectrometry) to analyse the semi-polar (secondary) metabolites. For the liquid samples, 300mg was extracted in 900ul solution (99.87% MeOH/0.13% formic acid). For powders or dry weight 30 mg was extracted in 1200 ul solution (75% MeOH/0.1% formic acid. After sonification and centrifugation, the samples were analysed on an LC-Orbitrap FTMS in positive and negative mode as described before (D'Urso et al, 2020; Campobenedetto et al, 2021).
The same samples were also analysed by GCMS (gas chromatography coupled to mass spectrometry to analyse mainly the polar (primary) metabolites. For the liquid samples 300 mg was extracted in 1200ul 100% MeOH. For the powders 20 mg was extracted in 1200ul 70% MeOH. After sonification and centrifugation the samples were analysed on GCMS with online derivatisation as described before (Pegiou et al, 2021). Untargeted data processing was performed using inhouse MetAlign-MetOT-MSClust workflow.

5.2 Results seaweeds and seaweed extracts (Olmix) 2019, 2020 and 2021

Figure 12 shows the principle component analysis (PCA) of four Seamel extracts and the 11 different seaweed species. The Seamel extracts are closely linked to (are most probably composed of) red seaweeds and *Ulva lactuca*, as can be seen by the red circle and the yellow circle.

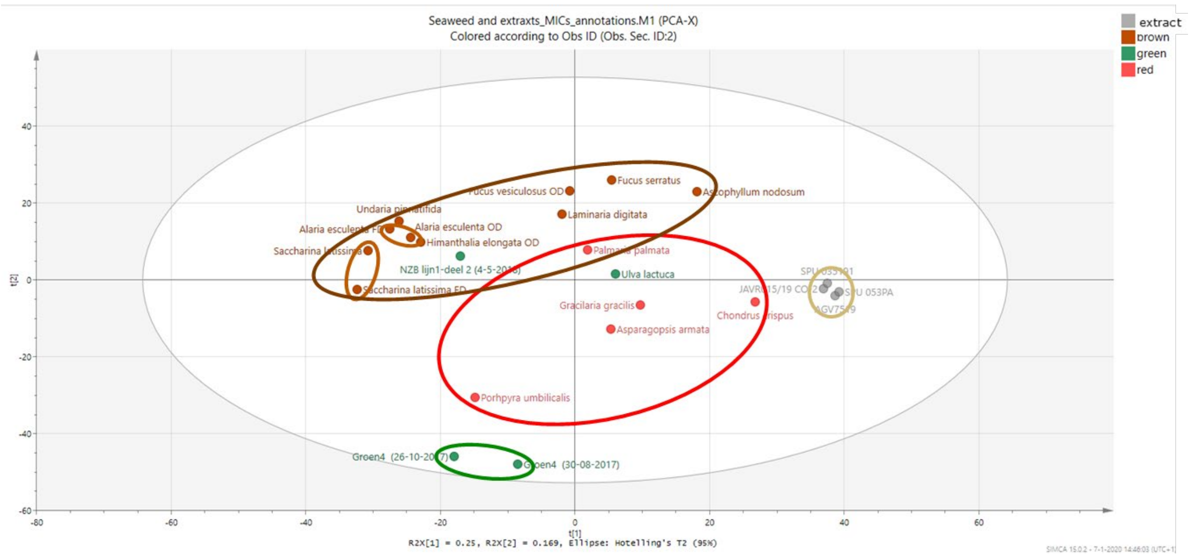

Figure 12 PCA analysis of four Olmix batches and 11 seaweed species, based on mass peaks analysed by LCMS.

Figure 13 shows the PCA analysis of all seaweed species, the Olmix samples and the other biostimulants analysed based on the metabolomics output (6095 LCMS mass peaks).
Even though all Seamel extracts do group together (yellow circle) there is variation between the batches and years which is almost as high as variation between species.
There is no overlap between species and the extracts, meaning that due to the extraction procedure a different (sub)set of metabolites is extracted compared to the intact species.

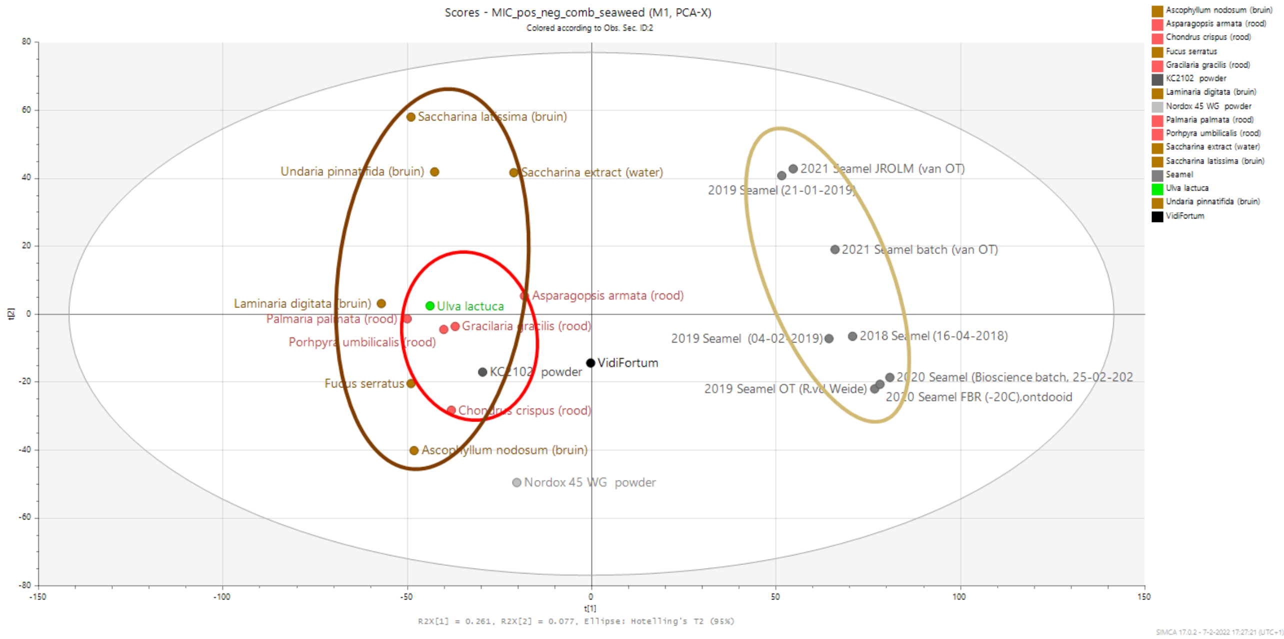

__Figure 13__ PCA analysis of all samples based on 6095 deduced mass peaks analysed by LCMS

When in a PCA only the different Seamel batches are compared based on the metabolites analysed by LCMS, it is clear that there is a large variation between the different years (in the direction of the Y axis) as can be seen in Figure 14, right circle. Separate of that group is a group of three batches, Seamel 2019 (21.01.2019), Seamel JROLM and Seamel 2021, at the left in the PCA. These are more correlated to each other than to the first circled group.

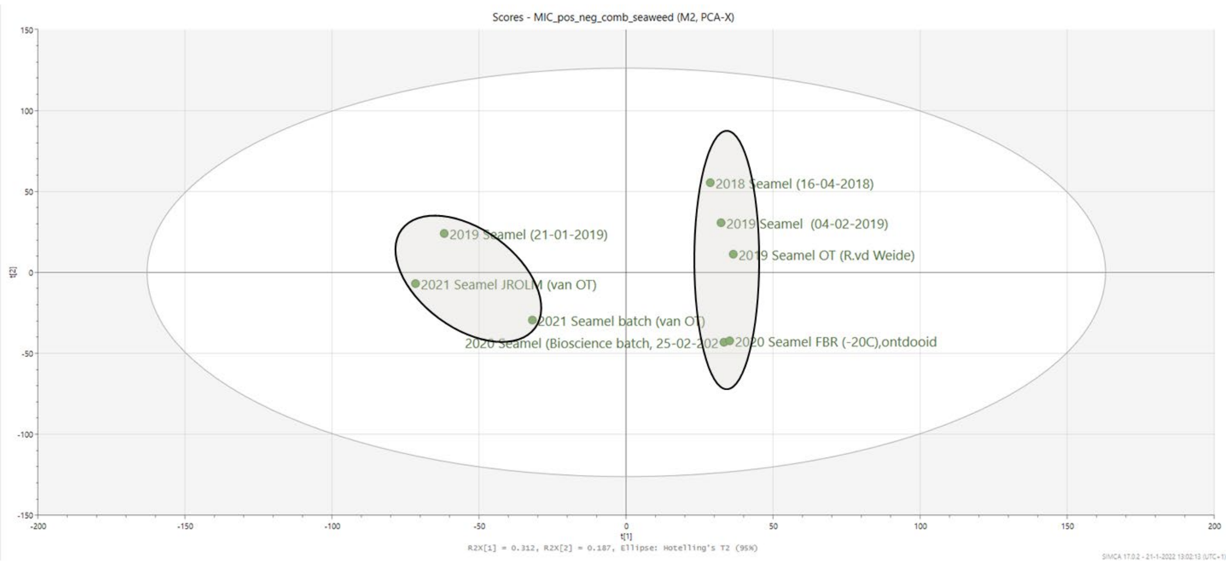

__Figure 14__ PCA analysis of all Seamel samples based on mass peaks analysed by LCMS

This variation is clear when looking at the chromatograms of the mass peaks as analysed by LCMS as can be seen in Figure 15. Especially in the green boxed regions of the chromatograms the differences in mass peaks is clear.

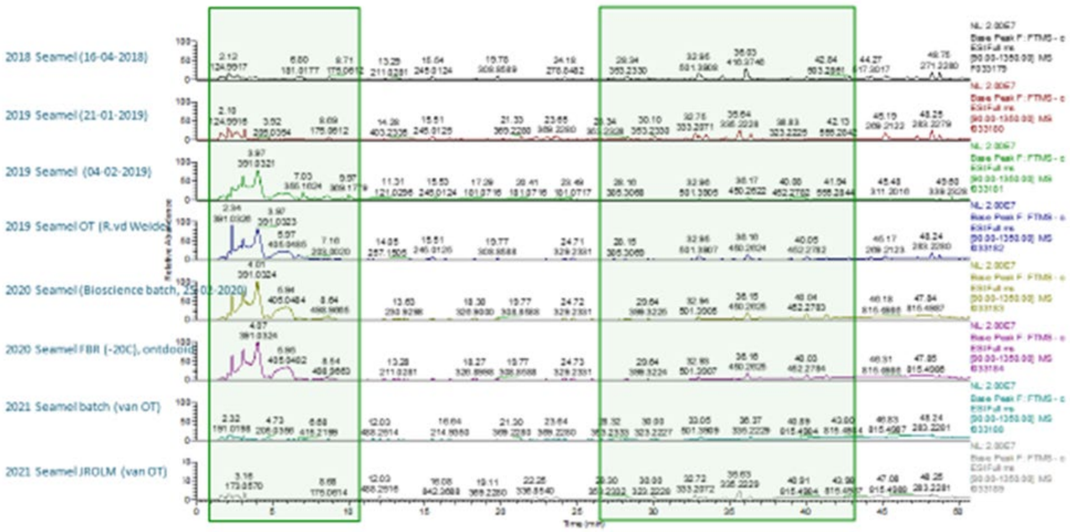

Figure 15 *LCMS profiles of all Seamel batches*

Analysing the more primary metabolites present in the extracts, using GCMS shows less variation between the batches, as can be seen in Figure 16. The Seamel extract in which no additives are present (JROLM) is clearly different from the other batches. Also the 2019 batch (04.02.2019) varies more from the other 2019, 2020 and 2021 batches.

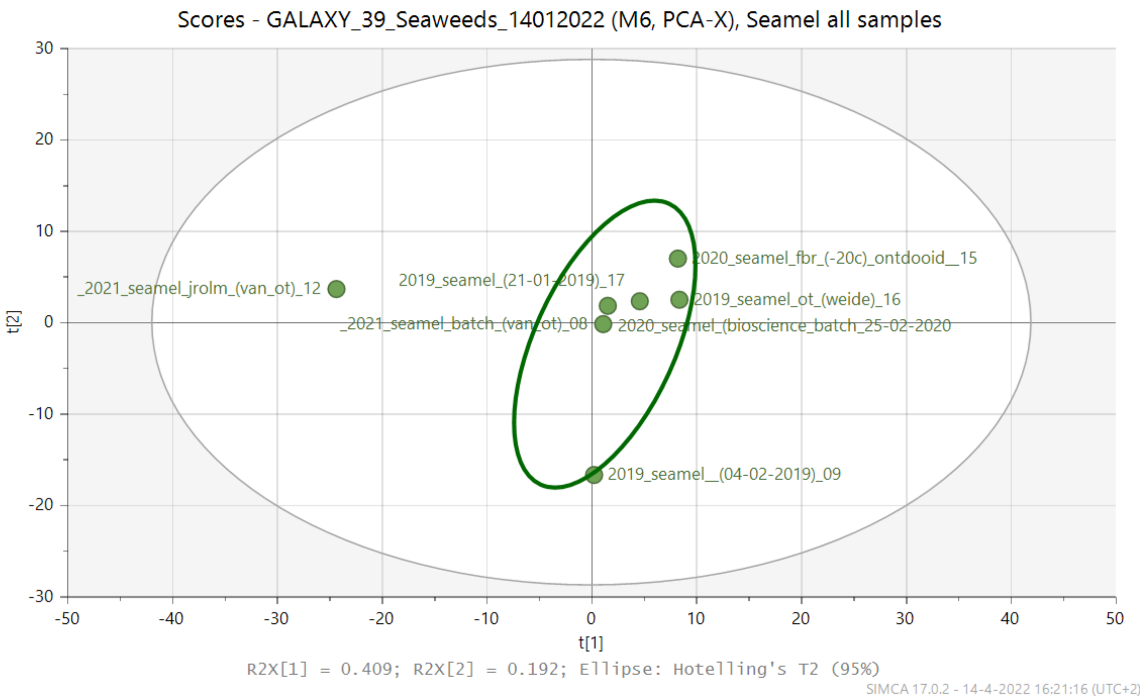

Figure 16 *PCA analysis of all Seamel samples based on mass peaks analysed by GCMS.*

5.3 Results seaweed extracts (Olmix) with/ without extra additives (JROLM)

The Seamel extract contains additional minerals next to an extract of seaweed plant material. Using LCMS and GCMS we analysed the difference between an extract with and without the added ‘ minerals’. Figure 17 shows the mass peaks of the two samples. It is clear that there seem to be more differences than only minerals that are present or absent. In the normal Seamel sample there is more citrate, formimino-glutamate, methylcitrate, propylmalate. On the other hand are there less oxidised omega-3 PUFAs.

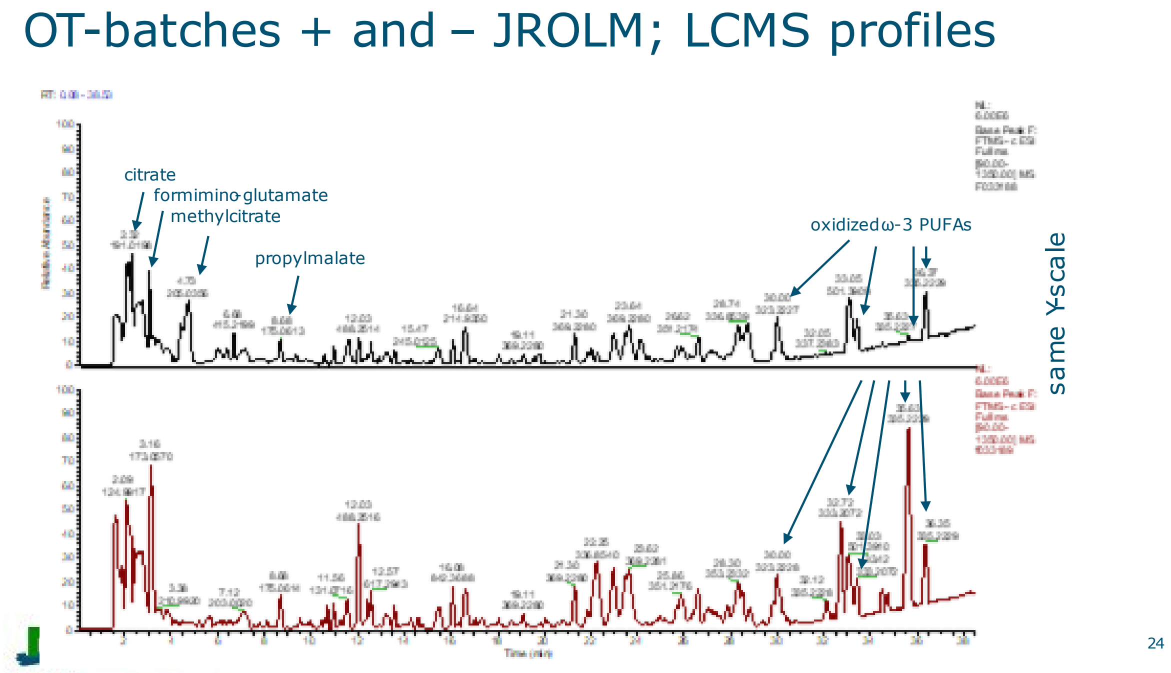

Figure 17 *LCMS analysis of Seamel sample with (upper chromatogram) and without additives (JROLM) (lower chromatogram).*

The same samples were also analysed by GCMS with which more primary metabolites can be detected.

Figure 18 shows the differences in GCMS detected metabolite peaks between the normal Seamel batch 2021 (OT) and the not-formulated JROLM batch in which no minerals have been added. Again the difference in citric acid is clear, and when zoomed in (Figure 19) there seems to be no fructose in the JROLM sample compared to the normal Seamel samples. Most differential metabolites in Seamel 2021 compared to Seamel 2021 JROLM are: glycolic acid, aconitic acid, d-Glucose, Benzoic acid L-hydroxy, Fructose, Citric acid, Aspartic acid and L-threonine.

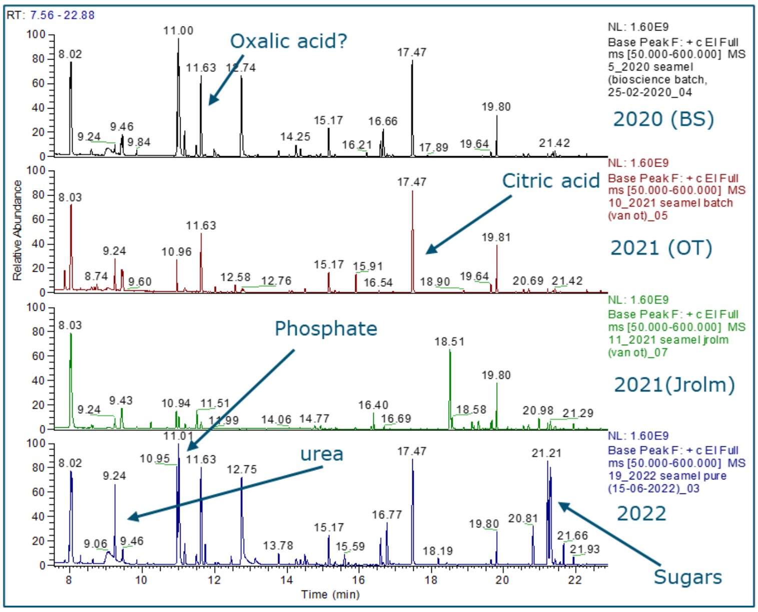

Figure 18 GCMS analysis of four Seamel samples (including JROLM).

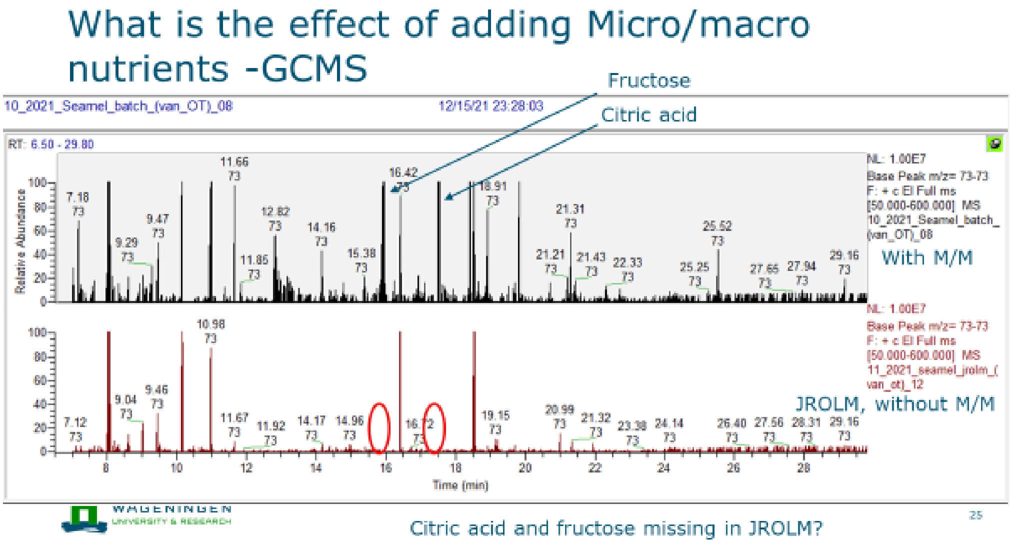

Figure 19 Zooming in the LCMS chromatogram of Seamel 2021 and the Seamel 2021 without added minerals (JROLM)

There is a clear difference between the normal Seamel 2021 batch and the batch that does not contain the additives (minerals) that are supplemented to the seaweed extract leading to the formulated commercial Seamel product. However, the differences do not point to only minerals that are varying. The differences might be caused by a different starting material from which both extracts were made. The JROLM batch did not result in positive effects in pot experiments as was described in 3.3.2.

5.4 Results Saccharina extract (W-FBR)

As was explained in 3.3.2, in a small side experiment an own produced (by Wageningen Food and Biobased Research) and not formulated extract from *Saccharina latissima* was tested. This extract was also analysed by LCMS and GCMS together with all other Seamel batches and seaweed species (Table 15). Figure 20 shows the PCA plot based on the metabolite profiles, in which the two yellow dots represent the Saccharina extract. The aqueous extracts are clearly different from the Saccharina seaweed starting material (blue circle for species and blue dots), and differ by metabolite composition from all Seamel batches (green dots).

However, this non-formulated aqueous extract was not effective in the pot experiments (3.3.2).

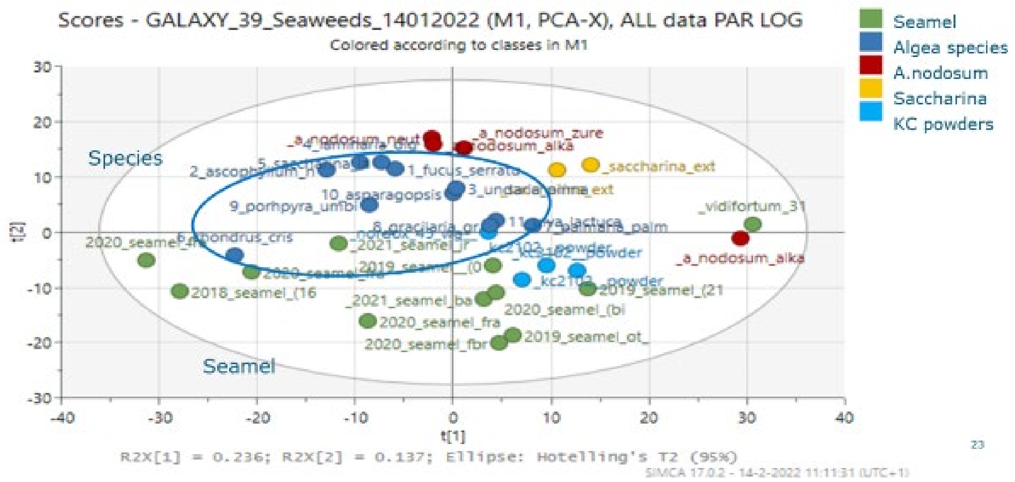

Figure 20 *PCA analysis of all samples based on mass peaks analysed by GCMS. In yellow the Saccharina watery extracts (W-FBR).*

5.5 Discussion and conclusion metabolomics analysis biostimulant extracts

Untargeted metabolomics analysis was performed using LCMS and GCMS on 11 different seaweed species, plus different Olmix Seamel Pure batches that were used in field- and pot experiments, a non-formulated Seamel batch (JROLM), a non-formulated aqueous Saccharina extract (from Wageningen Food and Biobased Research) and several other control commercial biostimulant extracts.

The following conclusions can be made:

- The Seamel batches are more related to red seaweed species and Ulva, and most probably consist mainly of red seaweeds and a lower percentage of green seaweeds.
- The seaweed species are separated, based on metabolic profile, from the Seamel and Saccharina extracts in a PCA plot. The extraction process extracts a subset of all metabolites present in the starting material. The metabolomics analysis could provide a long list of metabolites (peak areas) that are over- or under represented in the extracts compared to the plant species.
- The Seamel batches over the consecutive years vary from each other based on the metabolite profiles. This is most probably due to a diversity in starting plant material from which the extracts are made.
- There was more variation in metabolite profile between the Seamel 2021 batch and the non-formulated Seamel 2021 batch (JROLM; without minerals) than could be expected. This might be due to a difference in starting material of both samples. The JROLM batch did not affect resistance in a pot experiment, but it cannot be related to the absence of only minerals. Many more metabolites were varying between these two samples. It cannot be concluded that the formulation (adding minerals) gives the positive biostimulant effect.
- The own non-formulated Saccharina aqueous extract (W-FBR) was clearly separated in a PCA plot from the starting material, and varying from all the Seamel extracts. The Saccharina extract did not affect biotic stress response in a pot experiment and no conclusions can be made on the cause of this. Both the extraction and the plant experiments need to be repeated.
- In order to zoom in and pinpoint the specific metabolites that have a bio-activity on resistance to biotic stress in the crops they are applied on, a next research step would be to fractionate a biostimulant extract and in that way separate specific groups of metabolites. These subsets of the Seamel extracts then need to be tested in a plant experiment as well as being analysed

using metabolomics profiling. This research will lead to candidate metabolites (biomarkers) that are important in the biostimulant effect the Seamel extracts can have on crops after application.

- The variance between the different Seamel batches over the years, substantiate the importance of having good 'bio-markers' that can be used to validate batches for their putative biostimulant activity before they leave the factory. These biomarkers are preferably metabolites in the extract that direct, or indirect, affect biotic stress resistance in the crops.

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Annex 1 Tables with researches on 6 types of seaweed components

Type 1: Plant growth regulators (plant hormones/phytohormones)

Compound or group of compounds	Seaweed source	Product	Reported action	References
- Abscisic acid - Auxins - Cytokinins - Gibberellins	- *Ascophyllum, Laminaria* - *Ascophyllum, Fucus, Laminaria, Macrocystis, Undaria* - *Ascophyllum, Cystoseira, Ecklonia, Fucus, Macrocystis, Sargassum* - *Cystoseira, Ecklonia, Fucus, Petalonia, Sargassum*			(Craigie 2011) (Tables 5, 6)
Auxins and auxin-like compounds	Several species	Div	Promotes rooting	(Sharma et al. 2014), Lavine (2015), (Khan et al. 2009), (Nabti et al. 2017)
IAA (Indole-3-acetic acid)	*Ascophyllum nodosum* a. o., *Ulva*			(Khan et al. 2009), (Górka and Wieczorek 2017)
IBA (Indole-3-butyric acid)	*Ulva* (and other green seaweeds)			(Górka and Wieczorek 2017)
Cytokinins	Several species	Div	Cytokinins stimulate protein synthesis, cell division and several other growth parameters, e.g. bud formation. (Craigie 2011) mentions some concentrations in seaweed extracts	(Sharma et al. 2014), Lavine (2015), (Nabti et al. 2017), (Craigie 2011)
Cytokinins		e.g. Kelpak	Also delay senescence by reducing degradation of chlorophyll a. o.; inhibit rooting.	(Stirk 2006)
Cytokinins, zeatin	*Durvillaea potatorum*	Seasol	Plant growth regulation	(Arioli et al. 2015), (Khan et al. 2009)
Zeatin-riboside	Div		Plant growth regulation	(Khan et al. 2009)
Dihydro-zeathin Dihydro-zeathin-riboside	Div Div			(Khan et al. 2009) (Khan et al. 2009)
Trans-zeatin				(Khan et al. 2009)
Trans-zeatin riboside Dihydro derivatives of the above				(Khan et al. 2009) (Khan et al. 2009)
Aromatic cytokinins, BAP (benzyl amino purine)				(Khan et al. 2009)
Topolin				(Khan et al. 2009)
Ethylene Gibberellins	 Several species	 Div	 Induced amylase activity, stem elongation a.o. ((Nabti et al. 2017) mentions especially high concentration in green and brown seaweeds)	(Khan et al. 2009) (Sharma et al. 2014), (Khan et al. 2009), (Nabti et al. 2017)
Gibberellins	*Ecklonia maxima*, *Ulva*	Kelpak		(Arioli et al. 2015), (Górka and Wieczorek 2017)
Abcsisic acid (ABA)	*Ecklonia maxima*, *Ulva*			(Arioli et al. 2015), (Nabti et al. 2017), (Górka and Wieczorek 2017)
Brassinosteroids				(Arioli et al. 2015)
Strigolactones Brassinosteroids	 *Ecklonia maxima*		 Plant growth regulation	(Arioli et al. 2015) (Arioli et al. 2015)
Strigolactones	*Ecklonia maxima*			(Arioli et al. 2015)
Kinetin	*Ulva a.o.*			(Górka and Wieczorek 2017) Table 3
Jasmonates	*Fucus*		Induce defence, increase senescence and tuber formation, inhibits growth	(Craigie 2011)

Hormones (IAA, IPA)	*Laminaria, Ascophyllum nodosum*		In general, extracts stimulated root growth, nutrition, esterase activity, and sugar content in maize with high variations	(Ertani et al. 2018) (Michalak et al. 2020)
Cytokinins, auxins	*Ascophyllum nodosum*		The plant growth stimulators increased marketable yield and fruit size in strawberry plants	(Roussos et al. 2009) (Michalak et al. 2020)
Auxins and cytokinins, phytohormones	*Ecklonia maxima, Ascophyllum nodosum*	Kelpak® and Goëmar BM86	Several effects on apples: sometimes fruit set was improved or the size of apples, or the distribution of apples in size classes	(Basak 2008) (Michalak et al. 2020)
Auxin, gibberellins, substances with a cytokinin-like activity, cytokinins, abscisic acid, 1-amino-cyclopropane-lcarboxylicacid		Goemar GA 14	The extract applied as a foliar spray increased fresh matter production of maize seedlings	(Jeannin et al. 1991a) (Michalak et al. 2020)
Phytohormones: auxins, cytokinins	*Ecklonia maxima* (Osbeck)	Kelpak	Foliar applications improved marketable yield and nutritional quality of tomato	(Colla et al. 2017a, Colla et al. 2017b) (Michalak et al. 2020)
Auxin, cytokinins, fucoxanthin, auxin, cytokinins, vitamin C		Fucox and Ecklonia	Enhancement of fruit length, width, size, fresh weight, peel thickness etc of sour orange	(Al-Musawi 2018) (Michalak et al. 2020)

Type 2: Quaternary ammonium and tertiary sulphonium molecules (osmo-protectants), N-containing compounds

Compound or group of compounds	Seaweed source	Product	Action	References
Quaternary ammonium molecules, betaines	*Ascophyllum, Fucus, Laminaria*		Osmoprotective function, probably enhanced chlorophyll content in tomato and delaying senescence). Stress-related and offers protection from stress	(Arioli et al. 2015), Lavine 2015, (Khan et al. 2009), (Nabti et al. 2017), (du Jardin 2015)
Proline	Brown seaweeds		Protective against heavy metals by chelation and antioxidant activity	(Arioli et al. 2015) (Nabti et al. 2017), (du Jardin 2015), (Jardin 2012)
Betaines and betaine analogues	Several species	Div	Protection of cells from osmotic stress and effect on chlorophyll content by less degradation of chlorophyll	(Sharma et al. 2014), (Nabti et al. 2017), (Battacharyya et al. 2015), (Stadnik and Freitas 2014b), (Chojnacka et al. 2012)
ABAB (γ-Aminobutyric acid betaine)				(Battacharyya et al. 2015), (Stirk 2006)
Glycine betaine			Induction of resistance	(Battacharyya et al. 2015) (Craigie 2011) (Stirk 2006)
Laminine				(Battacharyya et al. 2015) (Stirk 2006)
δ-aminovaleric acid betaine				(Battacharyya et al. 2015) (Stirk 2006)
Lysine-betaine				(Stirk 2006)
Ascophylline				(Stirk 2006)
Tertiary sulphonium, dimethylsufoniopropionate (DMSP)	Several species		Osmoprotectant	(Nabti et al. 2017)
Hydroxy amides, culerpenyne/caulerpicin	*Caulerpa vanbosseae*		Antibacterial	(Kulik 1995)
Polyamines	Brown seaweed		Influence growth	(Craigie 2011)
Amino acids	*Ulva lactuca*			(Bikker et al. 2016) Table 3
Amino acids and vitamins	Several species		Support beneficial soil microbes	(Sangha et al. 2014)
Amino acids and derivatives, glutamate, histidine, proline, glycine betaine			Act as plant biostimulant and can be taken up by roots and through leaves	(Calvo et al. 2014)
Betaines (γ-aminobutyric acid betaine, δ-aminovaleric acid betaine, glycinebetaine)	*Ascophyllum nodosum*, *Laminaria digitata*, *L. hyperborea* and *Fucus serratus*		Increased chlorophyll content in several plant species	(Blunden et al. 2010) (Michalak et al. 2020)
Betaines	*Ascophyllum nodosum*		The plant growth stimulators increased marketable yield and fruit size in strawberry plants	(Roussos et al. 2009) (Michalak et al. 2020)
Betaines	*Ascophillum nodosum*	Actiwave®	Increased plant biomass in strawberries, fruit production, vegetative growth, leaf chlorophyll content, stomata density, photosynthetic rate and berry weight	(Spinelli et al. 2010) (Michalak et al. 2020)
Amino acids	*Ecklonia maxima*, *Ascophyllum nodosum*	Kelpak® and Goëmar BM86	Several effects on apples: sometimes fruit set was improved or the size of apples, or the distribution of apples in size classes	(Basak 2008) (Michalak et al. 2020)
Betaines		Goemar GA 14	The extract applied as a foliar spray increased fresh matter production of maize seedlings	(Jeannin et al. 1991b) (Michalak et al. 2020)

Proteins	*Ulva lactuca*		Enhance seed germination rates, higher production of mung beans, higher protein contents of seedlings	(Castellanos-Barriga et al. 2017) (Michalak et al. 2020)
Amino acids	*Ecklonia maxima* (Osbeck)	Kelpak	Foliar applications improved marketable yield and nutritional quality of tomato.	(Colla et al. 2017a, Colla et al. 2017b) (Michalak et al. 2020)
Proteins	*Ulva lactuca, Caulerpa sertularioides, Padina gymnospora* and *Sargassum liebmannii*		Induced protection against the necrotrophic fungus *Alternaria solani* in tomato plants	(Hernández-Herrera et al. 2014a, Hernández-Herrera et al. 2014b) (Michalak et al. 2020)
Amino acids		Fucox and Ecklonia	Enhancement of fruit length, width, size, fresh weight, peel thickness etc of sour orange	(Al-Musawi 2018) (Michalak et al. 2020)

Type 3: Alginate and several polysaccharides (or glycans), some sulphated, and their breakdown products (see also Table 2 from (Khan et al. 2009))

Compound or group of compounds	Seaweed source	Product	Action	References
Alginate and diverse polysaccharides, some sulphated			Stimulates root growth, induces plant genes involved in pathogenesis-induced defence	(Arioli et al. 2015)
Alginate and diverse polysaccharides, some sulphated			Trigger plant defence	(Arioli et al. 2015), (Burketova et al. 2015) (Stadnik and Freitas 2014a)
Carrageenan	Red seaweeds		Higher tolerance to *Sclerotinia* and plant defence responses in *Arabidopsis* by activation of jasmonic acid related genes	(Bulgari et al. 2015), (Stadnik and Freitas 2014b)
Laminarin/laminaran	*Laminaria digitata*		Induces plant defence response to *Botryrus cinerea* and *Plasmopora digitata* in grapevine	(Bulgari et al. 2015), Lavine, 2015
Laminarin/laminaran and carrageenan	Div		Especially carrageenan elicits an array of plant defence responses in tobacco	(Mercier et al. 2001), (Vera et al. 2011)
Alginates, laminarans, sulfated fucans and complex mucilages	Brown seaweeds		Multi defence responses in *Alfa alfa* en tobacco	(Khan et al. 2009)
Alginates and oligo-alginates	Brown seaweeds		Several effects, for example effective protection against tobacco mosaic virus tobacco plants	(Vera et al. 2011)
Polyuronides, alginates	Div		Induction of oxidative burst in plants; boost microbiology and used for soil remediation	(Sharma et al. 2014), Lavine, 2015, (Khan et al. 2009)
Alginates	Div		Trigger growth beneficial soil microbes and arbuscular mycorrhiza	(Khan et al. 2009), (Chojnacka et al. 2012)
Alginates	Brown seaweeds		Water holding compound in soil	(Nabti et al. 2017)
Fucans and oligofucans	Brown seaweeds		Triggers defence systems	(Vera et al. 2011), (Stadnik and Freitas 2014b)
Agars, carrageenans	Red seaweeds		Elicitors of defence	(Khan et al. 2009)
Mucilages with rhamnose, uronic acid, xylose	Green seaweeds		Induces defence by expression of PR-10 gen	(Khan et al. 2009)
(Oligo) carrageenans	Red seaweeds		Induces plants defence	(Shukla et al. 2016)
(Oligo) ulvans	*Ulva* sp.		Induces plant defence	(Walters et al. 2013)
Ulvans, uronic acid and sulphated rhamnose	*Ulva armoricana*		Induces plant defence	(Jaulneau et al. 2011)
Ulvans	Green seaweeds		Induce plant defence mechanisms	(Vera et al. 2011)
Ulvans (consisting of rhamnose, xylose, glucose, uronic acid, iduronic acid, sulfate, manose and galactose)	*Ulva*		Induces plant defence	(Vázquez-Rodríguez and Amaya-Guerra 2018), (Stadnik and Freitas 2014b)
Laminarin/laminaran	Brown seaweeds		Induces plant defence by activation salicylic, jasmonic acid or ethylene signalling pathway	(Sharma et al. 2014) Table 3, (Stadnik and Freitas 2014b)
Mannitol	Brown seaweeds		Chelating agent in soil	(Sharma et al. 2014), (Battacharyya et al. 2015)
Polyuronides, fucans or fucoidans	Brown seaweeds		Defending plants from microorganisms	(Sharma et al. 2014), Lavine, 2015, (Khan et al. 2009), (Chojnacka et al. 2012)
Fucose containing glucans				(Battacharyya et al. 2015)
Lichenan-like glucans			Stimulates root growth	(Battacharyya et al. 2015)
Oligosaccharides, polysaccharides	*Ascophyllum nodosum*	Stimplex™	enhanced disease resistance against e.g. *Alternaria* and *Fusarium* in cucumber probably through induction of defense genes or enzymes	(Jayaraman et al. 2011) (Michalak et al. 2020)

Polysaccharides (ulvans)	*Ulva armoricana*		Elicitation of a reporter gene regulated by a defence-gene promoter in a transgenic tobacco line, and Protection of cucumber plants against powdery mildew infection	(Jaulneau et al. 2011) (Michalak et al. 2020)
Polysaccharides	17 Moroccan seaweeds		Some had a beneficial effect on germination, plant biomass, and chlorophyll content of tomato	(Mzibra et al. 2018) (Michalak et al. 2020)
Laminarin	*Laminaria digitata*		efficient elicitor of defense responses in grapevine cells and plants and to effectively reduce *B. cinerea* and *P. viticola* development on infected grapevine plants	(Aziz et al. 2003) (Michalak et al. 2020)
Polysaccharides	*Sargassum latifolium*. *Hydroclathrus clathratus* and *Padina gymnospora*		Varying decreases of four soil borne plant pathogenic microbes: bacteria *Ralstonia solanacearum* and *Pectobacterium carotovora* and fungi *Fusarium solani* and *Rhizoctonia solani.* In some cases increases of eggplant yields	(Ibraheem et al. 2017) (Michalak et al. 2020)
Alginic acid	*Ascophyllum nodosum*	Actiwave®	Increased plant biomass in strawberries, fruit production, vegetative growth, leaf chlorophyll content, stomata density, photosynthetic rate and berry weight	(Spinelli et al. 2010) (Michalak et al. 2020)
Polysaccharides	*Laminaria digitata, Undaria pinnatifida, Porphyra umbilicalis*, *Eucheuma denticulatum* and *Gelidium pusillum*		Dose effect of treatments with an increase of fruit decay inhibition and reduction of disease severity in some tests with strawberries, peaches and lemons	(De Corato et al. 2017) (Michalak et al. 2020)
Poligosaccharides	*Ecklonia maxima*, *Ascophyllum nodosum*	Kelpak® and Goëmar BM86	Several effects on apples: sometimes fruit set was improved or the size of apples, or the distribution of apples in size classes	(Basak 2008) (Michalak et al. 2020)
Mannitol, laminaran, fucoidan, alginates		Goemar GA 14	The extract applied as a foliar spray increased fresh matter production of maize seedlings	(Jeannin et al. 1991b) (Michalak et al. 2020)
Carbohydrates	*Ulva lactuca*		Enhance seed germination rates, higher production of mung beans, higher protein contents of seedlings	(Castellanos-Barriga et al. 2017) (Michalak et al. 2020)
Carbohydrates	*Ecklonia maxima* (Osbeck)	Kelpak	Foliar applications improved marketable yield and nutritional quality of tomato	(Colla et al. 2017a, Colla et al. 2017b) (Michalak et al. 2020)
Carbohydrate	*Ulva lactuca, Caulerpa sertularioides Padina gymnospora, Sargassum liebmannii*		Induced protection against the necrotrophic fungus *Alternaria solani* in tomato plants	(Hernández-Herrera et al. 2014a, Hernández-Herrera et al. 2014b) (Michalak et al. 2020)
Uronic acid, fucose, laminarin, mannitol	*Ascophyllum nodosum*		Provide different levels of tolerance to drought stressed tomato plants	(Goñi et al. 2018) (Michalak et al. 2020)

Type 4: Micronutrients (e.g. minerals, trace elements, vitamins)

Compound or group of compounds	**Seaweed source**	**Product**	**Action**	**References**
Trace minerals/elements and nutrients			Plant nutrition	(Arioli et al. 2015) and (Sharma et al. 2014)
Macro and micro elements and toxic metals	Mix of *Ulva*, *Polysiphonia*, *Cladophora*		Not specified	(Godlewska et al. 2016)
K, Na, Ca, Mg, Zn, Cu, Cl, S, P, Va, Co, Mn, Se, Br, I, As Fe, F			Some essential for crop growth	Lavine, 2015
Different minerals			Provide part of essential nutrients for plants, improve mineral uptake by plant roots and leaves	(Khan et al. 2009)
Cu, Co, Mn, Fe	*Laminaria* a.o.			(Craigie 2011)
Vitamins A, C, E	*U. rigida*			(Vázquez-Rodríguez and Amaya-Guerra 2018)
C, N, K, Ca, Fe, Mn	Mix of Brown and red seaweed species *Ascophyllum nodosum, Chondrus crispus, Fucus vesiculus, A. nodosum, Fucus* sp.		Similar or more K, Ca, S and Fe compared to land plants. Increased soil salinity.	(Possinger and Amador 2016)
C, N, Na, Mg, K, Ca, P, Fe, Al, Mn, B, Sr, Zn, Cu, Se, Cr, Ba, Ni, V, As, Co, Pb, Mo, Cd, Hg	*Ulva ohnoi*		Used for making compost mixes with bagasse. Mn, Zn and Bo are important for sugarcane production, but high ECs (e.g. caused by Na) can be disadvantageous	(Cole et al. 2016) Table 1

Cu, Mn, Zn, Fe, K, Mg, Co, Cr, Pb, Ni, Cd, Na, Ca	*Lithothamnion calcareum, Ulva lactuca, Stoechospermum marginatum*		In green seaweed much Na, but less Na in red and brown seaweeds (other mechanism)	(Nabti et al. 2017) Table 1
N, P, Ca, Mg, K, Na, Fe	*Ulva lactuca*		High variation in concentrations	(Breure 2014) Table 2
P, Ca, K, Mg, Na, Cl, S, Cu, Fe, Mn, Zn, Ni, As, Co, Se, Cd, Pb, Hg	*Ulva lactuca*			(Bikker et al. 2016) Table 2
N, P, K, Ca, Fe, Mg, Zn, Na, S	*A. nodosum*		When applied on leaves, nutrient uptake can be increased in plants	(Battacharyya et al. 2015)
Ca, Mg, Na, K, Fe, P, Cl, SO_4, Si, Zn, Cu, NO_3	*Sargassum wightii* and *Caulerpa chemnitzia*		Promoted seedling growth of *Vigna sinensis* beans, e.g. shoot and root length, fresh and dry weight, chlorophyll, carotenoids, shoot protein content	(Sivasankari et al. 2006) (Michalak et al. 2020)
Kahydrin (derivative of vit K1)	*Ascophillum nodosum*	Actiwave®	Increased plant biomass in strawberries, fruit production, vegetative growth, leaf chlorophyll content, stomata density, photosynthetic rate and berry weight	(Spinelli et al. 2010) (Michalak et al. 2020)
N, Mg, B	*Ecklonia maxima*, *Ascophyllum nodosum*	Kelpak® and Goëmar BM86	Several effects on apples: sometimes fruit set was improved or the size of apples, or the distribution of apples in size classes	(Basak 2008) (Michalak et al. 2020)
Minerals	*Ulva lactuca*		Enhance seed germination rates, higher production of mung beans, higher protein contents of seedlings	(Castellanos-Barriga et al. 2017) (Michalak et al. 2020)
Vitamins: B1, B2, C, E, Elements: N, P, K, Ca, Mg, Fe, Mn, B, Zn, Cu	*Ecklonia maxima* (Osbeck)	Kelpak	Foliar applications improved marketable yield and nutritional quality of tomato.	(Colla et al. 2017a, Colla et al. 2017b) (Michalak et al. 2020)
Macroelements	*Ulva lactuca* and *Caulerpa sertularioides*, *Padina gymnospora* and *Sargassum liebmannii*		Induced protection against the necrotrophic fungus *Alternaria solani* in tomato plants	(Hernández-Herrera et al. 2014a, Hernández-Herrera et al. 2014b) (Michalak et al. 2020)
Vitamin C		Fucox and Ecklonia	Enhancement of fruit length, width, size, fresh weight, peel thickness etc of sour orange	(Al-Musawi 2018) (Michalak et al. 2020)
Sulfate	*Ascophyllum nodosum*		Provide different levels of tolerance to drought stressed tomato plants	(Goñi et al. 2018) (Michalak et al. 2020)

Type 5: Lipid based molecules (see also Table 3 below from (Khan et al. 2009))

Compound or group of compounds	Seaweed source	Action	References
Sterols			(Craigie 2011)
Sterols: fucosterol and derivates	Brown seaweeds		(Khan et al. 2009) Table 3
Cholesterol and derivates	Red seaweeds		(Khan et al. 2009)
Ergosterol, 24-methylene cholesterol	Green seaweeds		(Khan et al. 2009)
Fatty acids, e.g. palmitic acid		Some with antimicrobial properties	(Sharma et al. 2014), (Hamed et al. 2018)
Fatty acids, mostly palmitic acid and isomers of C18:1	*Ulva lactuca*		(Bikker et al. 2016) Table 4
Fatty acid derivative and oxylipins	*Acrosiphonia coalita*	Antibacterial properties	(Kulik 1995)
Fatty acids	*Sargassum latifolium*. *Hydroclathrus clathratus* and *Padina gymnospora*	Varying decreases of plant pathogenic microbes: bacteria *Ralstonia solanacearum* and *Pectobacterium carotovora* and fungi *Fusarium solani* and *Rhizoctonia solani.* In some cases increases of eggplant yields	(Ibraheem et al. 2017) (Michalak et al. 2020)
Fatty acids	*Laminaria digitata, Undaria pinnatifida, Porphyra umbilicalis*, *Eucheuma denticulatum* and *Gelidium pusillum*	Dose effect of treatments with an increase of fruit decay inhibition and reduction of disease severity in some tests with strawberries, peaches and lemons	(De Corato et al. 2017) (Michalak et al. 2020)

Type 6: Secondary metabolites

Compound or group of compounds	Seaweed source	Product	Action	References
Brominated phenolic derivatives	*Polysiphonia lanora*		Antibacterial effects	(Bajpai 2016)
Anthraquinons	*Laurencia spectabilis*		Possibly antibacterial effects	(Bajpai 2016)
Halimedatrial and caulerpenin	Green seaweeds		Ecological role in defense and signalling	(Pereira and Costa-Lotufo 2012)
Lanosol, vidalol, elatol	Red seaweeds		Ecological role in defense and signalling	(Pereira and Costa-Lotufo 2012)
Dictyopteren C, pachydictyol A, fucodiphlorethol, epitaondiol	Brown seaweeds		Ecological role in defense and signalling	(Pereira and Costa-Lotufo 2012)
Several compounds			Antiviral and antifouling properties	(Pereira and Costa-Lotufo 2012) Tables 1 and 2
Halogenated furanones and enones	*Delisea pulchra*		Alter pathogenicity of bacteria, lactone regulation, antifouling	(Khan et al. 2009), (Hellio et al. 2000)
Phlorotannins (polyphenols)	Brown seaweeds		Antifouling, antioxidant	(Hellio et al. 2000), (Battacharyya et al. 2015), (Craigie 2011), (Chojnacka et al. 2012)
Phloroglucinol, eckol, dieckol, fucols	Fucaceae			(Craigie 2011) Table 11
Brominated phenol lanosol (phlorotannins)	*Rhodomela larix*		Antifouling	(Hellio et al. 2000)
Bioflavonoids rutin, quercetin, kampherol	*Gracilaria dendroides*, *Ulva reticula*, *Dyctyota ciliolate*		Antimicrobial	(Bajpai 2016)
Sesquiterpenoid hydroquinones peyssonic acid A and B	*Peyssonnelia* sp		Inhibit growth of some bacterial and fungal pathogens	(Bajpai 2016), (Chojnacka et al. 2012)
Sesquiterpenoid hydroquinones tiomanene & acetylmajapolene	*Laurencia* sp.		Antimicrobial	(Bajpai 2016)
Zonarol and isozonoral sesquiterpenes	*Dictyopteris zonarioides*		Inhibiting effect on plant pathogenic fungi	(Bajpai 2016)
Terpenes			Antimicrobial activities	(Nabti et al. 2017)
Pigments (terpenes), carotenoids			Protect plants from chlorophyll degradation	(Chojnacka et al. 2012)
Gloiosiphones A en B	*Gloiosiphonia verticillaris*		Antibacterial properties	(Kulik 1995)
Phenolic compounds	*Ascophyllum nodosum*		Stimulation of flavonoid synthesis in spinach leaf thus enhancing its nutritional quality	(Fan et al. 2011) (Michalak et al. 2020)
Phenolics: phenolic acids (gallic, protocatechuic, vanillic, caffeic, p-coumaric, syringic, p-hydroxybenzoic)	*Laminaria* and *Ascophyllum nodosum*		In general, extracts stimulated root growth, nutrition, esterase activity, and sugar content in maize with high variations	(Ertani et al. 2018) (Michalak et al. 2020)
Phenolic acids, flavonoids	*Sargassum vulgare*		All extracts showed antifungal activity against *Pythium aphanidermatum* in potato	(Ammar et al. 2017) (Michalak et al. 2020)
Phenolic compounds	*Sargassum latifolium*. *Hydroclathrus clathratus* and *Padina gymnospora*		Varying decreases of four soil borne plant pathogenic microbes: bacteria *Ralstonia solanacearum* and *Pectobacterium carotovora* and fungi *Fusarium solani* and *Rhizoctonia solani.* In some cases increases of eggplant yields	(Ibraheem et al. 2017) (Michalak et al. 2020)
Phenolic compounds, phlorotannins	*Laminaria digitata, Undaria pinnatifida, Porphyra umbilicalis*, *Eucheuma denticulatum* and *Gelidium pusillum*		Dose effect of treatments with an increase of fruit decay inhibition and reduction of disease severity in some tests with strawberries, peaches and lemons	(De Corato et al. 2017) (Michalak et al. 2020)
Phenolic compounds		Goemar GA 14	The extract applied as a foliar spray increased fresh matter production of maize seedlings	(Jeannin et al. 1991b) (Michalak et al. 2020)
Total phenols	*Ulva lactuca*, *Sargassum filipendula* and *Gelidium serrulatum*		Reduced disease severity of *A. solani* and *X. vesicatoria* coupled with elevated rates of activities of defence enzymes in tomato	(Ramkissoon et al. 2017) (Michalak et al. 2020)
Fucoxanthin		Fucox and Ecklonia	Enhancement of fruit length, width, size, fresh weight, peel thickness etc of sour orange	(Al-Musawi 2018) (Michalak et al. 2020)
Polyphenols, chlorophyll, carotenoids	*Polysiphonia*, *Ulva* and *Cladophora*		Enhanced chlorophyll and carotenoid content in plant shoots, as well as root thickness and above-ground biomass of garden cress (*Lepidium sativum* L.; Brassicaceae) and wheat (*Triticum aestivum* L.; Poaceae)	(Michalak et al. 2016b) (Michalak et al. 2020)

Polyphenol	*Ascophyllum nodosum*	Provide different levels of tolerance to drought stressed tomato plants	(Goñi et al. 2018) (Michalak et al. 2020)

Annex 2 Links to the complete field and pot trial reports

Field experiments in onion

Geel, W.C.A. van, Evenhuis, A. en Topper, C.G., 2020. Effect humuszurenproduct en zeewierextract bij uien. Verslag van een veldproef in 2019 te Lelystad. Wageningen Research, rapport WPR-870 https://edepot.wur.nl/541283.

Geel, W.C.A. van, Evenhuis, A. en Topper, C.G., 2019. Effect humuszuurproduct en zeewierextract bij uien. Verslag van een veldproef in 2018 te Lelystad. Wageningen Research, rapport WPR-869 https://edepot.wur.nl/541282.

Object 2018	**Object 2019**	**Description**	
A	AB	Reference untreated	Fertilizer NPK
Q	F	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha just before bulbing and two weeks later second treatment
R	G	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha just before bulbing and two weeks later second treatment
S	H	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha in the 3-leave stage and just before bulbing

Greenhouse experiments with tomato

2018

A. Evenhuis & C.G. Topper, 2019. *Biological efficacy of a biorationals based on sea weed and humic acid to control potato late blight.* Wageningen Research, Report WPR-OT-936 https://edepot.wur.nl/570732

Table 20 *Treatments and fungicides applied 1 or 7 days before inoculation.*

Code	**Fungicide**	**Active ingredient**	**Dose rate l or kg per ha**	**Spray application**
A	UTC	-	-	-
B	Dithane DG NT	mancozeb 750 g/kg	2.0	- 1 day
P	Seamel Pure		1.0	- 1 day
R	Seamel Pure		2.0	- 1 day
T	Seamel Pure		2.0	-7 days

2021

A. Evenhuis & C.G. Topper, 2021. *Biological efficacy of experimental products to control potato late blight.* Wageningen Research, Report WPR-OT-937 https://edepot.wur.nl/570733

Table 21 Treatments and fungicides applied 7 (T1) and 1 (T2) day before inoculation.

Code	Fungicide	Dose rate l or kg per ha	Active ingredient	Spray applications
A	UTC	-	-	-
B	Nordox 45 WG	0.5	Cu 450 g/kg	T2
C				
D	Seamel	2		T1 & T2
E	Seamel	2		T2
F	Seamel	1		T2
G	Saccharina	12.5		T1 & T2
H	Saccharina	12.5		T2
J	Saccharina	6.25		T2
K	JROLM	2		T2
L				
M	UTC not inoculated	-		-

Field experiments in potato
2019

Evenhuis, A. en Schepers, H.T.M.A., 2019. *Efficacy to control potato late blight by applying biological crop protection products* Wageningen Research https://edepot.wur.nl/541281

Object	Description	
A	Reference untreated	
E	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 0.5 L/ha weekly spraying start at12 June till 21 August
F	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha weekly spraying start at12 June till 21 August
G	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha weekly spraying start at12 June till 21 August

2020

Evenhuis, A., 2020. *Efficacy to control potato late blight by applying biological crop protection products* Wageningen Research Report WPR-OT-938 https://edepot.wur.nl/570734

Object	Description	
A	Reference untreated	
C	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 0.5 L/ha weekly from 10 Jun to 24 August
D	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha weekly from 10 Jun to 24 August
E	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha weekly from 10 Jun to 24 August
F	JROLM extract (Seamel without additional minerals) Foliar spraying:	Foliar 2 L/ha weekly from 10 Jun to 24 August

2021

Evenhuis, A., 2021. *Efficacy to control potato late blight by applying biological crop protection products* Wageningen Research Report WPR-OT-939 https://edepot.wur.nl/570735

Object	Description	
A	Reference untreated	
B	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 2 L/ha weekly from 28 Jun to 9 August
L	Seaweed extract (Seamel Pure) Foliar spraying:	Foliar 1 L/ha weekly from 28 Jun to 9 August

To explore the potential of nature to improve the quality of life

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Report WPR-OT-940

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