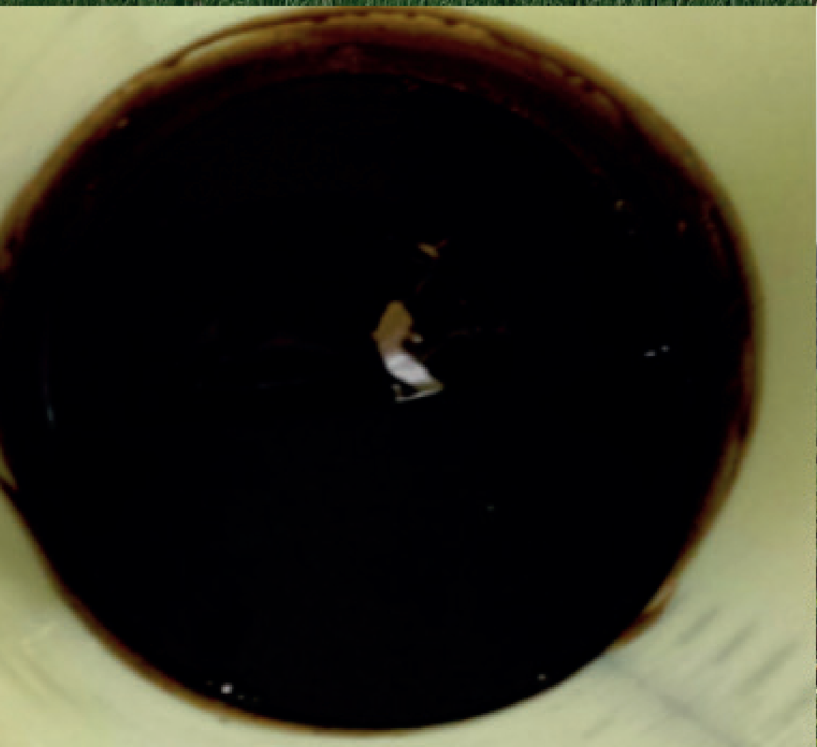

Prospects for Humic Acid Products from Digestate in the Netherlands

Quickscan

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The goal of this research was to examine the prospects for humic acid products and in particular a humic acid product produced from animal manure/digestate. Based on a literature review it is concluded that humic acids are used worldwide as biostimulants and overall positive effects on crop growth are reported, but variation in results is high. Stakeholders explained during interviews that in the Netherlands the use of Humic acid products are mostly related to niche markets. For conventional agriculture in the Netherland the value of humic acids has not been proven yet, this can be explained by the high fertility of the Dutch soils, and high yields that are already obtained without adding humic acids. Also field experiments were done. In several field experiments with potatoes and onions the specific humic acid product was tested. Adding the humic acids (on soil and as foliar spraying) did not benefit crop growth, but also did not harm the crops. No effect was found on the development of diseases.

Keywords: Humic acids, animal manure, digestate, biostimulant

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Photo cover: Luuk Gollenbeek

Contents

Preface

In the Netherlands, there are surpluses of manure and digestates/biogas slurries (digested manure and residual flows), which represent a negative value. At the same time, these residual flows contain valuable ingredients for the production of biomass (as raw material for food and feed products), for improving soil quality and for energy production. The number of feasible business cases in which the residual flow is upgraded has so far been limited. This is due both to the efficiency of the technologies used and the legislation and regulations related to the residual flows.
Recent information from research, scientific literature and companies provides new starting points for a biobased valorization of manure/digestate streams and improving the efficiency of anaerobic digestion. The innovative aspect of our research is the cultivation of new types of biomass on the residual flows and the use of the conversion products to improve anaerobic digestion. This involves the use of separated manure and digestate products for the cultivation of mushrooms/fungi, worms, insects, specific bacteria and aquatic biomass. The resulting biomass can be further refined and marketed as food, feed and bio-based feedstock. There are also processed manure and digestate products that are valuable as fertilizer products for soil and plant growth, as substrate for improvement of anaerobic digestion or for export/use besides in agriculture. This gives a new interpretation to obligatory manure processing.
The aim of this project is to further explore and substantiate/test these ideas on lab and practical scale, leading to a proof of principles for new bio-based upgrading methods for manure and digestate that can be used in conjunction to better close cycles and/or sell outside regular agriculture. Bottlenecks in legislation and regulations are explored and put on the agenda. Key figures are also calculated that are necessary for assessing sustainability (e.g. costs, environmental effects) and for supporting legislation (e.g. minerals, food safety).
The livestock sector gains insight into the possibilities of biobased valorisation and better marketing of their most important residual flows. For the SMEs involved, this research provides proof of principle for their technology and input in their business cases. The combined effects of the technologies provide new knowledge, methods and research directions for science. In a social context, the use and upgrading of manure and digestates in other ways also contributes to the transition to a circular bioeconomy
with an efficient and sustainable agri-food sector.
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Summary

The Wageningen Research conducted a Quickscan to examine the prospects for products comprising humic acids, such as the humic acid product produced by Ecoson.

The research questions were:

- What are the characteristics of the humic acid products now on the market?
- Which crops can these humic acid products be used for and what doses and times of application are used?
- What is the use of humic acids in practice?
- Which stakeholders are interested in using humic acid products and what further information and actions are needed?

To answer these research questions, a desk study was carried out, a literature research was conducted and five stakeholders were interviewed, also several field studies were carried out to research the effect of humic acids on crop yield and quality.

Humic and fulvic acids are fractions of organic compounds with similar characteristics, these terms originate from soil science. Humic acid products that are on the market in the Netherlands are derived from brown coal (lignitite/leardonite) or are produced by decolouring drinking water (Vitens). In research also humic acids from compost are used, and the extraction of humic acids from animal manure/digestate by Ecoson is a new source. In terminology humic acids, fulvic acids and humic substances are commonly exchanged and used for marketing purposes.

Humic acids are used as biostimulants and as food and feed additives. Most perspective for the humic acid product from Ecoson is the use as a biostimulant considering the law and regulations for animal manure. There are several mechanisms of humic acids described that can stimulate plant growth. For instance increase of availability of soil phosphorus/nutrients, increase of water holding capacity of the soil, hormonal like reactions in the plant, enhanced growth of root system. A strong variability in the effects of humic acid products on crop growth is found, the effect depends on the source of the humic substances, the environmental conditions, the receiving plant, the dose and the manner in which the humic acid products are applied. On average, yield increases are reported by al kinds of crops (onion, wheat, potato, strawberry, maize, grass, grapes). Field trials conducted in the Netherlands are sparse this might be due to the optimal conditions that already exist.

In literature it was found that application rates used are within the range 1.5 to 20 kg humic substances/ha. Optimal application rates depend on humic substances product and crop. Application rates of circa 8 kg humic substances/ha are thought to be an adequate guideline. In the Netherlands grass and maize, followed by potatoes, wheat and sugarbeet are the main crops produced considering use of land. So these the use of humic acids for these crops can lead to a potentially big market for humic acids. If profit per hectare is the main selection criteria red cabbage, strawberries, fruit growing and potatoes are promising for the use of humic acids. These crops represent a smaller market but because of higher profits the use of humic acids can be more economically beneficial.

Several experiments with the humic acid product from Ecoson were conducted. The humic acid product was tested in an onion field trial; no significant positive or negative effect on yield and quality was found. A field trial with potatoes did also give no significant positive or negative effects on yield. For both field experiments disease pressure was registered, but probably due to the hot and dry summers of 2018 and 2019 disease pressure was low. The Humic acids of Ecoson can be sprayed with normal field sprayers in doses of 120 l Humic acids of Ecoson/hectare. Crop spraying with doses 1,5-3 l Humic acids of Ecoson/hectare per spraying (two times spraying) seemed safe for onion, potatoes and tomato plants.

From interviews with stakeholders it was concluded that humic acids will not have an added value for regular cultivation of crops in the Netherlands. Maybe within the biological cultivation of crops the use of humic acids can give an advantage. Only when costs will decrease and additional yields are proven,

humic acid products can be successful. In soil based horticulture humic substances are used. Maybe a role of humic acids can be the replacement of iron chelates that are now used in the irrigation water in non-soil based horticulture for the complexation of metal ions. These iron chelates are relatively expensive but a stable system is more important than costs of fertilizers. For pear trees the humic acids are advised to use to prevent pear decline. The fruit sector can be a relevant market for humic acids. For all sectors the benefits of humic acids should be proven in field trials (in the Netherlands) to persuade the farmers to start applying them.

Marketing of humic acids requires further proof of product. Field experiments are needed followed by word-of-mouth advertising. Not only the farmers should be persuaded but also the advisors or fertilizer suppliers.

1 Introduction

Wageningen Research aims to develop nature-based processes to increase the value of manure and digestate. Therefore knowledge and power are bundled with a consortium of partners within the project 'Biobased valorisation of manure and digestate'. Darling Ingredients ('DarlingI') is one of the partners and wants to focus on humic acids (humic acid, fulvic acid) extracted from digestate. DarlingI has developed a new biobased technology and is able to extract the humic acids (see Figure 1). DarlingI now wants to explore the market for these products.

The goal of this Quickscan is to examine the prospects for products comprising humic acids, such as that produced by DarlingI.

The research questions are:

- What are the characteristics of the humic acid products now on the market (Chapter 3)?
- Which crops can these humic acid products be used for and what doses and times of application are used (Chapter 4)?
- What is the use of humic acids in practice (Chapter 5)?
- Which stakeholders are interested in using humic acid products and what further information and actions are needed (chapter 6)?

To answer these research questions, a desk study was carried out, a literature research was conducted and five stakeholders were interviewed. The effect of humic acid products on the quality and quantity of onions was evaluated in a field study and compared against findings in a field study on potatoes carried out by DarlingI in cooperation with Wageningen Research. Furthermore, a greenhouse trial was conducted to investigate the effect of leaf application of a humic acid product on the development of an important *Phythophthora* disease.

Figure 1.1 Humic acids of Ecoson, an extracted humic acid product developed by DarlingI. Photo: Wageningen Plant Research.

2 Information on humic substances

2.1 Humic substances

Soil consists of a mineral fraction, an organic fraction, water and air (soil pores). Soil scientists define a specific group of organic compounds in the soil as humic substances. These include humic and fulvic acids, and also hydrophilic acids and hydrophobic neutral organic matter. Using extraction methods, humic and fulvic acids can be separated out. The components are discriminated by behaviour (for extraction scheme, see Appendix 1 to this report) (Van Zomeren en Comans 2007). These humic and fulvic acids are known to be complex structures of organics with relatively high amounts of aromatic structures, and are also known to be resistant to degradation. It is hypothesised that these compounds are created during degradation processes in the soil, but this hypothesis has been challenged by Lehmann and Kleber (2015), who suggest that the humic and fulvic acids detected in measurements might be an artefact of the analytical process (i.e. that acid and alkaline extraction could induce their creation). However, there is no doubt that some soil characteristics (higher cation exchange capacity (CEC), higher soil water-holding capacity, lower degradation rate of organic matter, lower leaching of nutrients) are associated with higher measured amounts of humic and fulvic acids in the soil.

In the Netherlands, different products based on humic acids and fulvic acids are available. These humic acid-based products are commonly used as a soil improver/plant biostimulant (https://www.triferto.eu/nl ; http://humifirst.be/). Other uses are:

- Feed additive: Agrivalid sells a feed additive containing humic acids and states that cows, pigs and horses can benefit (http://www.agrivalid.eu/nl/sectoren/feed)
- Food additive/health improver: For instance, the company Health Solutions specialises in producing humic and fulvic acids for health and food/feed applications (https://health-solution.eu/nl/)
- Technical applications/research; Humintech (Germany) produces humic acid-based products for removal of metals from water or concrete liquefier (https://www.humintech.com/industry.html).

2.2 Plant biostimulants

The definition of plant biostimulants according to the European Biostimulant Industry Council (EBIC, http://www.biostimulants.eu/, 2016) is:
"Materials which contain substance(s) and/or microorganisms, whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independent of its nutrient content. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides."
Biostimulants can have different physiological effects on plants (see Figure 2.1).

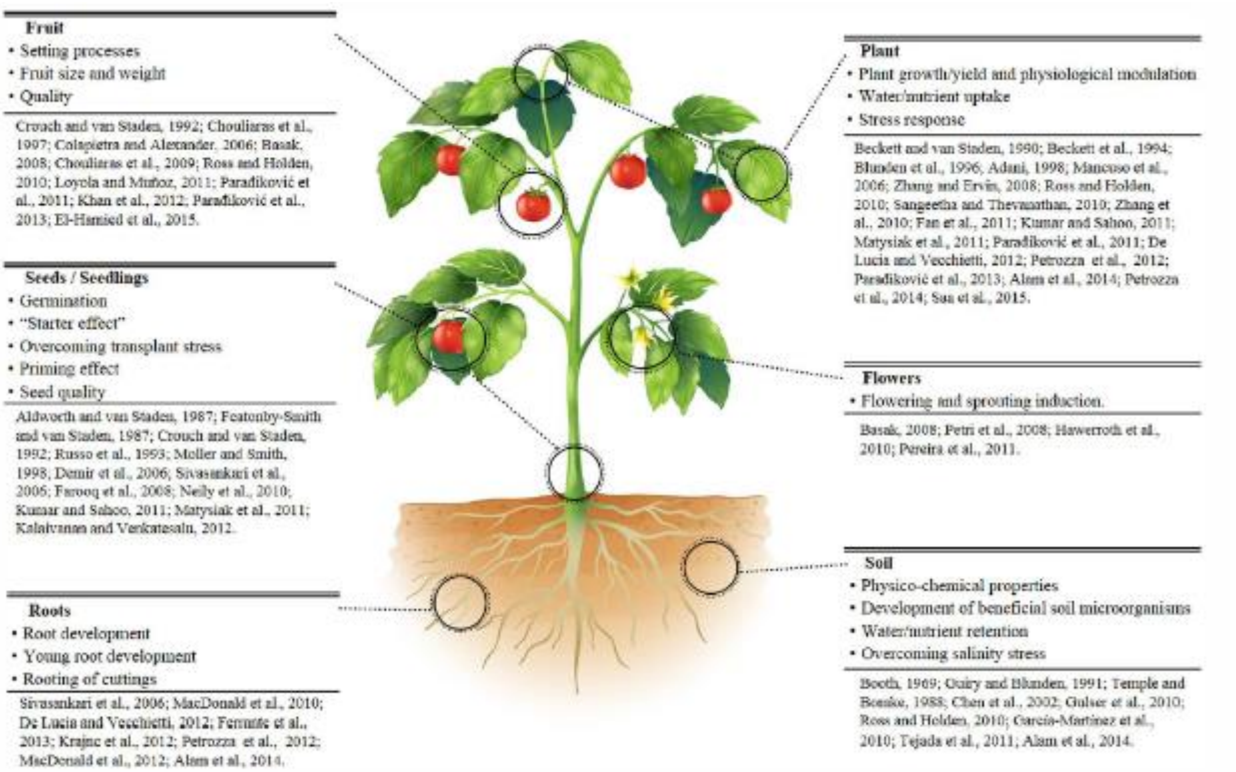

Figure 2.1 Reported examples of the main effects and physiological actions played by plant biostimulants (PBS). Source: Povero G, Mejia JF, Di Tommaso D, Piaggesi A and Warrior P (2016) A systematic approach to discover and characterize natural plant biostimulants. Front. Plant Sci. 7:435. doi: 10.3389/fpls.2016.00435

Du Jardin (2015) categorised plant biostimulants into:

- Humic and fulvic acids
- Protein hydrolysates and other nitrogen-containing compounds
- Seaweed extracts and botanicals
- Chitosan and other biopolymers
- Inorganic compounds
- Beneficial fungi
- Beneficial bacteria

Biostimulants are not captured in EC regulations (either in EC 1107/2009 Plant Protection Products or in EC 2003/2003 Fertilisers). Humic acids of Ecoson, the product developed by DarlingI, is derived from animal manure and will keep the status of animal manure under the current regulations. Animal manure and products from animal manure are not permitted for use as a feed additive under EU Regulation 767/2009, article 6, Appendix 3, 1[1]. The main future use of Humic acids of Ecoson is expected to be as a plant biostimulant. Therefore this report focuses on use of humic acid and fulvic acid products (hereafter 'humic acids products') as plant biostimulants, and not in health applications.

2.3 Plant biostimulants humic substances

For humic acid products, a series of mechanisms leading to increasing plant growth have been described. These comprise: increased bioavailability and uptake of nutrients as an effect of increased CEC (Du Jardin 2015), increased water-holding capacity of the soil (Piccolo et al. 1996), stimulation of plasma

[1] Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC (Text with EEA relevance);

membrane H^+ ATPases, increasing nutrient uptake (Du Jardin 2015), hormone-like reactions (suggested by Atiyeh *et al.* 2002) and various effects on microorganisms (Hasset et al. 1986; Sharif et al. 2002; Siddiqui et al. 2009).

Rose et al. (2014) reported strong variability in the effects of humic acid products and concluded that the effect is dependent on the source of the humic substances, the environmental conditions, the receiving plant, the dose and the manner in which the humic acid products are applied. Du Jardin (2015) suggested that the different results of studies can also be (partly) attributed to the complex dynamics of humic and fulvic acids in the soil by forming supramolecular colloids, influenced by exudates of plant roots. Rose et al. (2014) carried out a meta-analysis of published studies on humic acid products and estimated an average positive effect on plant yield of 15-25%. Those authors also identified the most important factors impacting the effect of humic acid products to be: the source of the humic/fulvic acids, plant type and stress conditions.

Humic and fulvic acids are extracted from different sources: peat, brown coal (lignite/leonardite), compost and soil (Du Jardin 2015). Relatively new sources are groundwater (Vitens), liquid manure (DarlingI) and wastewater (Stichting Toegepast Onderzoek Waterbeheer (STOWA)).

Humic acid products as a plant biostimulant are used in different forms: solution, powder, coating of artificial fertiliser and seed coating. Note that humic acid products are also present naturally in compost and animal manure.

For this research it is important to understand that humic acids and fulvic acids are fractions of organic compounds with similar characteristics and that these terms originate from soil science, the composition of humic acids can differ between different sources. In terminology humic acids and humic substances are commonly exchanged and used for marketing. Effects measured with humic substances differ because of different circumstances, overall positive effects on plant growth are found.

3 Inventory of available humic acid products

The properties of three humic acid products available for use as a biostimulant, including Humic acids of Ecoson, are shown in Table 3.1. The focus is on their biostimulant function because this application is the only one available for Humic acids of Ecoson under current EU regulations. As can be seen from Table 3.1, for most products only minimal data on their properties are published on websites and in scientific articles. Although commercial claims about these products can be found, the analytical background is generally not presented. Based on these minimal data presented in table 3.1 the concentrations of humic and fulvic acids in Humic acids of Ecoson are lower than in the other available products. This is not a problem for their effect as a biostimulant, because all products are diluted when applied, but it means that the transport costs can be higher for Humic acids of Ecoson. Compared with the product Humic, the amounts of salts in Humic acids of Ecoson are in the same range (based on the limited data in Table 3.1). The market value of the products will be set based on field trials and the experiences of users. Moreover, as stated in Chapter 2, the quality of the humic and fulvic acids can differ between products.

In order to be able to compare more parameters of the humic acid products a sample of Humic acids Ecoson and another product on the market 'product X' were analysed by Koch-Eurolab. In table 3.2 the results of this analyses is shown (see also appendix 4).
The amounts of humic acids and fulvic acids measured are much lower (roughly 3 times) than the amounts expected (table 3.1 and 3.2). Causes of this discrepancy can be differences in practise of analytical methods or differences in quality between badges of humic acids. The test was only performed on one sample per product. So statistically the result can only be used as a indication of the content of the humic acid products.

The product of Ecoson has got much higher amounts of Copper, Nickel and Zinck than the Product X. These high levels of metals might become a problem if new European legislation is made about biostimulants.
For Product X remarkable high levels of Phosphor, Potassium and Sodium were reported, these levels were also higher than found in the Ecoson product (P factor 15 K factor 5 Na factor 5). On the other hand nitrogen is a factor 9 higher in the Ecoson product.

Table 3.1 Available humic acid products in the Netherlands, composition according to companies

Product name:	Humic acids Ecoson[1]	HumiFirst[2] DRIP	Humic[3]
Manufacturer (source)	Ecoson (animal manure)	Tradecorp (lignite)	Vitens/Triferto (drinking water)
Price (euro/L)	nb	4	
Price (euro/g)	-	-	-
Use	Soil improver	Soil improver	Soil improver
Dry matter content (%)	19-21		22
Organic matter content (% of dry matter)	80		12
Humic acids (g/kg)	50	64	250
Fulvic acids (g/kg)	19	192	
pH	8.8 - 9.2	7	8.1
N (g/L)	18		
P (P_2O_5) (g/L)	1.3		
K (g/kg)	11		
Cl (g/kg)	0.5		2.4 (as NaCl)
Na (g/kg)	3.4		2.4 (as NaCl)
Cd (mg/kg)	<0.1		
Cu (mg/kg)	37		
Pb (mg/kg)	<2		
Zn (mg/kg)	119		

[1]*Information provided by DarlingI.* [2]*Information from* www.humifirst.be. [3]*Combined information from* http://www.triferto.eu/nl/home *and Sjoerdma* et al *(2013).*

Table 3.2 Results chemical analyses Koch-Eurolab Humic acids Ecoson and product X. In gram per kg product or in milligram per kg dry matter

Product name:	Humic acids Ecoson	Product X
Dry matter content (g/kg)	122	290
Organic matter content (g/kg)	70.4	137
Humic acids (g/kg)	10.5	6
Fulvic acids (g/kg)	2.4	13.9
pH	8.4	6.83
N (g/kg)	12.4	1.4
P (P_2O_5) (g/kg)	2.0	31.2
K (g/kg)	9.4	50.3
Cl (g/kg)		
Na (g/kg)	3.4	16
Cd (mg/kg dm)	1.0	0.1
Cu (mg/kg dm)	605	6.9
Ni (mg/kg dm)	110	6.6
Pb (mg/kg dm)	4	1.7
Zn (mg/kg dm)	1762	22

4 Potential uses of humic acid products

4.1 Potential uses of humic acid products for field crops

Calvo et al. (2014) inventoried different possible agricultural uses of humic acid products. A table from Calvo et al. (2014) that summarises almost 50 studies (1980 to 2013) on the use of humic acid products in different cultivations can be found in Appendix 2. Based on that review, it can be concluded that many scientific peer-reviewed studies have been conducted to examine the effects of humic acid products on plant growth, but many of these studies have been carried out in the laboratory or on pot plants in growth chambers. The effects observed in these conditions can be very high and not representative of the practical situation. Therefore literature about field studies was collated and is summarised in Table 4.1. Humic acid products are applied by foliar spraying and soil application, and positive and negative results have been found for both forms of application. Application rates used in field experiments are within the range 1.5 to 20 kg humic substances/ha. In Table 4.2 for the selected crops, the number of hectares and the average profit per hectare in the Netherlands is also included, to give an indication of the possible market and possible economic effect for commercial humic acid products in the Netherlands.

Table 4.*1* Different uses of humic acid products for field crops world-wide and in the Netherlands

Crop	Appl-ication	Application rate (kg humic/fulvic acid/ha)	Study conditions, country	Effect (humic substances)	References
Onion	Soil	20	Field, India	Yield +11%	Sangeetha and Singaram (2007)
Sugar beet	Foliar	-	Field Egypt	HA[#] Yield +14% FA[##] Yield +23%	Hassanin et al. (2016)
Wheat					?
Common bean	Foliar	-	Field, Egypt	Yield +10%	Ibrahim et al. (2012)
Broccoli	Soil	-	Field, Egypt	Yield +15%	Selim and Mosa (2012)
Potato	Soil	8.2 and 16.4	Field, Egypt	Water stress: no effect. Without water stress: increased yield	Selim et al. (2012)
	Soil	3.9 to 8.25	Field, Belgium	Yield +13 and +17 %	Verlinden et al. (2009)
Grassland	Soil	3.5 to 15	Field, Belgium	Yield -8 and +10 %	Verlinden et al. (2009)
Maize	Soil	8.25	Field, Belgium	Yield 0 and +2%	Verlinden et al. (2009)
	Soil (row)	1.5	Field, Belgium	Yield +1 and +3 %	Verlinden et al. (2009)
Strawberry	Foliar	-	Field, Italy	Yield -23%, better quality	Neri et al. (2002)
	Soil	-	Greenhouse, Iran	Yield +47 and +103 %,	Escghi et al. (2015)
Fruit growing					?

#HA=humic acid, ## FA= fulvic acid, - rate per hectare unknown, ? no relevant article found.

Table 4.2 *Hectares of field crops in the Netherlands and average unit revenues and yields.*

Crop	Effect Humic substances	1000 ha *	Revenue Euro/hectare**	Profit Euro/hectare**	Yield** Tonnes /hectare
Onion	Yield +11%	25	5,000 – 5,950	2,897 – 2,107	50.5-59.5
Sugar beet	HA# Yield +14% FA## Yield +23%	71	3,435 – 3,831	2,143 – 2,481	75.1 – 83.7
Wheat	?	128	1,885 – 2008#	1,118 – 1,170#	8.5 – 8.9#
Common bean	Yield +10%	1	2,345	1,134	3.3
Broccoli	Yield +15%	nb	16,720 - 15,200	14,113	30,400 (numbers)
Potato	Yield +0 and +13 and +17 %	157	7,300 - 11,172	4,442 – 7,589	39.9 – 52.5
Grassland	Yield -8 and +10 %	936	2,736***	1,964***	7,6 dm***
Maize	Yield 0 and +3%	206.9	2,063	1,095	16.5 dm
Strawberry	Yield -23% +47 and +103	2.8	38,285##	20,473##	20,1##
Fruit Growing	?	20	27,000###	5,000###	40###
Red Cabbage	?	0,5	27,500	19,238	55,0

*From CBS statline amount of hectares cultivated in the Netherlands in 2016. ? no relevant article found. ** source KWIN AGV 2018. *** Data applies for seed and hay production, grassland used for dairy cattle will have a higher production of dry matter, but a lower financial yield. dm = dry matter. #Based on Winter wheat. ## Based on open field cultivation. ### Calculated values from data available at www.agrimatie.nl.

4.2 Experiments with field crops

As can be seen from the area used for different crops (Table 4.1), grass, maize, potato and wheat are the main crops in the Netherlands. Verlinden et al. (2009) investigated the effect of humic acid products on maize, grass and potatoes in Belgium and their results can be representative (in terms of climate, soil use, soil quality) for the situation in the Netherlands. Their experiments were carried out in field situations and in pot experiments. Only results of the field experiments are summarised in this report. The product HumiFirst was used for the experiments and a dose of 8.25 kg/hectare was applied. Two forms were tested: liquid humic/fulvic acids and incorporated humic/fulvic acids (fertiliser coated with humic/fulvic acids).

For grassland (permanent and new grassland), Verlinden et al. (2009) found that the first yield after humic acid application was higher than in the control (12-16% for liquid application and 3-42% for humic acid-incorporating product. For the whole season, grass yields in these two treatments were similar to that in the control (-8% to +10% in yield, respectively). For both broadcast and row application, only small effects were obtained. According to Verlinden et al. (2009), this can be due to the high nutrient levels in the soil. The highest increase in yield was obtained for potatoes (+13 and +17 % for low and high application rate, respectively). Based on the results of statistical analysis, Verlinden et al. (2009) concluded that the increases and decreases in yield on a yearly basis were not statistically significant.

The study by Selim et al. (2012) referred to in Table 4.1 was conducted in Egypt and the results are thus less representative for the Netherlands. Under water stress conditions, application of humic acid products in that study did not have an effect on potato tuber yield, while under normal water conditions application of humic acid products led to an increase of 19% in tuber yield, with the highest yield for a high application rate of 16.4 kg humic acids/hectare.

For wheat, no relevant published field study was found. However, a summary of five studies about humic acid products and wheat (pot, growth chamber and hydroponic studies) is presented in Appendix 2. Positive results on growth were found in these studies, but a field test in one study found no increase or a decrease in the growth of wheat.

The crops sugar beet, onion and strawberries and several vegetables are also grown on an reasonable area in the Netherlands and, because of their higher selling price, information stated in Table 4.1 for these crops could also be very relevant. The studies summarised in Table 4.1 found positive effects of humic acid products on yield for all these crops. However, it should be borne in mind that these studies were conducted under different conditions from those in the Netherlands.

Tomatoes, peppers and cucumbers cultivated in greenhouses are other potential crops on which humic acid products can be used. The different studies listed in the table in Appendix 2 show positive results in glasshouse production of these crops following addition of humic acid products to the nutrient solution.

Literature research about the use of humic substances in fruit growing was found (for instance apples, pears, olives and abricots). Some studies showed benefits for fruit growing, but set ups of these experiments did not give relevant information for the Dutch situation.

4.3 Application rate

Dobbs et al. (2010) found that the optimal dose varied depending on the humic acid/s used and also the plants to which they were applied. There is an indication that if growth-stimulating hormones are available above a certain level, plant growth will decrease following application of humic acid products. This might be the reason why there is an optimal dose for humic acid products.
For HumiFirst, a dose of 50 litres per hectare is advised for soil application. This is equivalent to an active dose of 8.3 kg humic/fulvic acids per hectare. This advice from the manufacturer of HumiFirst is in line with the dosages reported in studies involving field experiments (see Table 4.1). No recent advice on application rates were found for the product Humic.

The effect of yearly application of humic acid products to the soil on crop yields and whether application rates should be altered with yearly use remain to be determined.

If we do a quick and dirty calculation based on assumptions: a sandy soil in the Netherlands with 5% soil organic matter, 5 gram (Spijker et al, 2009) humic substances per kg soil, an A horizon of 0.25 m and a soil density of 1.6 kg/litre.
In this case per m^2 soil 2 kg humic substances are present in the A horizon=> 5 * (1*1*0.25*1.6)

Applying a dose 8 kg of humic acids per hectare gives a doses of 0.0008 kg/m^2. So this is circa 0.04 % of the humic acids content already present in the soil.

Based on this quick and dirty calculation the amounts of humic substances added to the soil is low compared to the humic substances already present in the soil.

5 Stakeholder vision

Five experts were interviewed to gain more information about the market conditions of humic acids in the Netherlands:

- Luuk Hagting (Agrifirm); fertilizers and biostimulants
- Wim Voogt (WUR); horticulture substrate cultivation
- Wim van Dijk (WUR); field crops
- Rien van der Maas (WUR); fruit growing
- Chris van Laarhoven (Tuinbouw advies); horticulture soil cultivation

The interviewed persons are not experts on humic acids but are well known with practices within their sector.

Field Crops
Known is that the humic and fulvic acids are produced with the degradation of organic materials. The humic acids have chelating properties and phosphate and other micro nutrients are more available for the crops. Also soil structure and water holding capacity of the soil are increased with humic acids. Brands that are known are Humifirst (Tradecorp) and Humic (Triferto).

The use of humic acid products in the Netherlands in field crops is low. A hypotheses is that the crops will especially benefit from humic acid products if the circumstances are suboptimal. In the Netherlands agriculture and fertilization is optimized. Maybe this is the reason why the positive effects of humic acids are not found in general during field trials in the Netherlands. In tests with other soil improvers like black carbon also less effect was found than during experiments in other countries. Also animal manure and other organic fertilizers or organic soil conditioners are used, which can also have (potential) humic acid in it. If humic acid products are used it can be as a liquid, a solid or as a coating.

An important factor to explain the use of humic acids is the relation between the fertilizer dealer/advisor and the farmer rather than crop use, soil type or region. So marketing of the dealer/advisor and experiences of a farmer with the product are key factors. The cost benefits are thought to be negative within the Netherlands because of the already optimal growing conditions (it will cost more to purchase and apply the humic acid products than the yield and profit will increase).

For regular cultivation of field crops in the Netherlands the experts think that humic acid will not have an added value. Maybe within the biological cultivation of crops the use of humic acids can give an advantage. Only when costs will decrease and additional yields are proven, humic acid products can be successful.

Horticulture
In the Netherlands Humic acids are almost not used in substrate horticulture, but in soil based horticulture they are. Main reasons not to use humic acids in substrate horticulture is that humic acids can lead to blockage and formation of biofilms in the irrigation system. And 85 to 90 % of the horticulture is done on substrates with drip irrigation. Also the effectiveness is not well proven and fertilization costs are secondary to the reliability of the fertilizer.

In soil based horticulture doses of 10-25 ltr /ha are used and well known products are Pow Humus and Humifirst. Pow Humus puts a lot of effort in creating a market for the humic substances but without success. Liquid products are preferred because these are easy to use also solid humic acid products are used. Important is also that it is thought that different humic acid products have different effectiveness and quality. The humic acids are used to improve the soil conditions by increasing the amount of fungi in the soil, to buffer an excess of Potassium or Sodium (resulting in a better uptake of Calcium and Manganese) and increases the bioavailability of Phosphate.

Maybe a role of humic acids can be the replacement of iron chelates that are now used in the irrigation water for the complexation of metal ions. These iron chelates are relatively expensive but a stable system is more important than costs of fertilizers.

Fruit
Humic acids are used for fruit trees. Humic acids are advised for pear trees by Fruit Consult and CAF (Centrale Adviesdienst Fruitteelt). It is advised to give a liquid fertilizer product of ammonia nitrate, iron (chelates) and humic acid for pear trees just before harvest. The humic acids are supposed to increase the roots system (and prevent Pear Decline) and can have an effect on the quality of the pears. In this

particular matter the Humic acids are advised and therefore used. For pears also benefits in pear color were determined by research in the Netherlands. A doses of 15 ml humifirst dissolved in 10 liters of water is advised especially for trees that show deficiency of nutrients. If all trees are treated a doses of up to 40 liters can be used based on this advice.
So the fruit sector can be a market for humic acids.

For all sectors the benefits of humic acids should be proven in field trials (in the Netherlands) to persuade the farmers to start applying them. Mikkelsen (2005) also concluded; on farm field trials are needed to determine effectiveness.

6 Experiments with humic acid products from digestate

6.1 Onion research

Introduction
In 2017, 2018 and 2019, a test on various potassium fertilisation strategies 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 potassium and method of application (via the soil or as a foliar treatment). An additional treatment was included to test the effects of the humic acid of Ecoson for 2018 and 2019. This was sprayed over the plants or applied to the soil. 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., 2020a en 2020b the reports are in Dutch and anonymised. In the paragraph below a summary of this research is given. The for humic acid relevant treatments are shown in table 1.

Table 6.1 Objects Humic acid product

Object 2018	Object 2019	Description	
A	AB	Reference untreated	Fertilizer NPK
N	C	Humic acid product Soil application:	100 L/ha before sowing
O	D	Humic acid product Foliar spraying:	Foliar 1,5 L/ha just before bulbing and two weeks later second treatment
P	E	Humic acid product Foliar spraying:	Foliar 3 L/ha just before bulbing and two weeks later second treatment

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 6.2). 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 6.3).

In 2019 on average 81 plants per m^2 were present. Only object C (Soil application of humic acids) 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. 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.

***Table 6.2** 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%

Tabel 6.3 *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%
N	Humic acid Soil	49,0	7,5	40,6	83%
O	Humic acid Foliar 1	45,4	7,1	35,6	78%
P	Humic acid Foliar 2	48,4	7,5	39,4	81%
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%
C	Humic acid Soil	75,3	10,8	61,9	82%
D	Humic acid Foliar 1	76,3	10,6	62,8	82%
E	Humic acid Foliar 2	76,6	10,6	64,2	84%
F pr.		*n.s.*	*n.s.*	*n.s.*	*n.s.*

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 6.4).

Table 6.4. *Hardness before and after storage (index) 2018*

Object	Desciption	Before storage	After storage
2018			
A	Reference	100	88
N	Humic acid Soil	101	93
O	Humic acid Foliar 1	96	89
P	Humic acid Foliar 2	103	88
F pr.		*n.s.*	*n.s.*
2019			
AB	Reference	99	59
C	Humic acid Soil	104	61
D	Humic acid Foliar 1	98	59
E	Humic acid Foliar 2	101	59
F pr.		*n.s.*	*n.s.*

In table 6.5 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 6.5. *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
N	Humic acid Soil	123	52	130	89	11	90
O	Humic acid Foliar 1	116	52	130	84	10	85
P	Humic acid Foliar 2	121	55	135	85	11	79
F pr.		*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
2019							
AB	Reference	161	61	171	98	18	9,7
C	Humic acid Soil	149	58	158	87	17	9,2
D	Humic acid Foliar 1	148	56	158	89	17	9,0
E	Humic acid Foliar 2	155	59	162	94	16	8,4
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..

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.

6.2 Potato field experiment

In 2018, an experiment with potatoes and addition of humic acid product was conducted in a potato field comprising 1 hectare. A brief description of the experiment is presented in Appendix 4. In brief, potatoes were grown with and without soil addition of the humic acid product of Ecoson at a rate of 120 l per hectare. Growth of the tubers was determined by visual inspection. Because of the dry summer of 2018, growth of the tubers was slow and the farmer and PEKA KROEF decided that sampling before harvest was not needed. Visually, there was no difference between development of tubers with or without humic acid product treatment
The research conducted was very basic but it can be concluded that:

- Humic acid product of Ecoson can be applied with normal tillage machinery
- In 2018, adding a humic acid product did not lead to differences in potato tuber growth, but this was only determined by a quick visual inspection.

In 2019 this research was not repeated.

6.3 Potato late blight research

2018 pot experiment tomatoes
A pot experiment with tomato plants was carried out to investigate the effect of humic acid product on late blight disease development. It was a first explorative study to . The tomato plants were grown in a greenhouse and were inoculated with late blight. The plants were then sprayed with different doses of humic acid product, another tested product 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.

Figure 6.1 *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.

2019 field experiment potatoes

In 2019 a field experiment was set up with potatoes. This research is reported in an anonymised report (Evenhuis and Schepers 2020). 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, EFGH testing of different products.
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.

Table 1. Objects Humic acid product

Object	Description	
A	Reference untreated	
B	Humic acid product Soil application:	100 L/ha before sowing
C	Humic acid product Foliar spraying:	Foliar 1,5 L/ha just before bulbing and two weeks later second treatment
D	Humic acid product Foliar spraying:	Foliar 3 L/ha just before bulbing and two weeks later second treatment

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 6.1 the effect of the different treatments can be seen for late blight development.

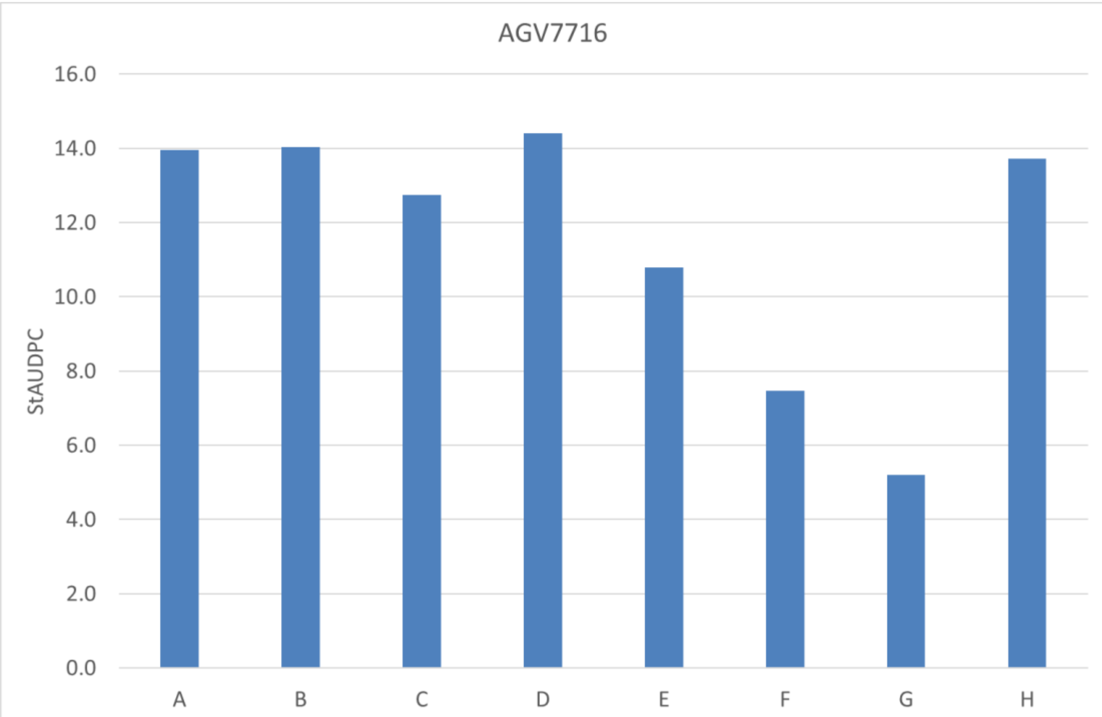

Figure 6.1 Potato late blight StAUDPC as a result of various spray schedules

Tuber blight incidence was low and based on weighed none of the treatments were significantly different from the untreated control. Weather conditions were not conducive for tuber blight since rain was limited and foliar blight severity remained low until half August.

Total yield was around 40 t/ha which was medium yield considering the dry season. Due to foliar blight severity increasing strong in the second half of August desiccation was carried out early September. Normally in September the crop could grow and a yield of around 60 t/ha would have been possible.

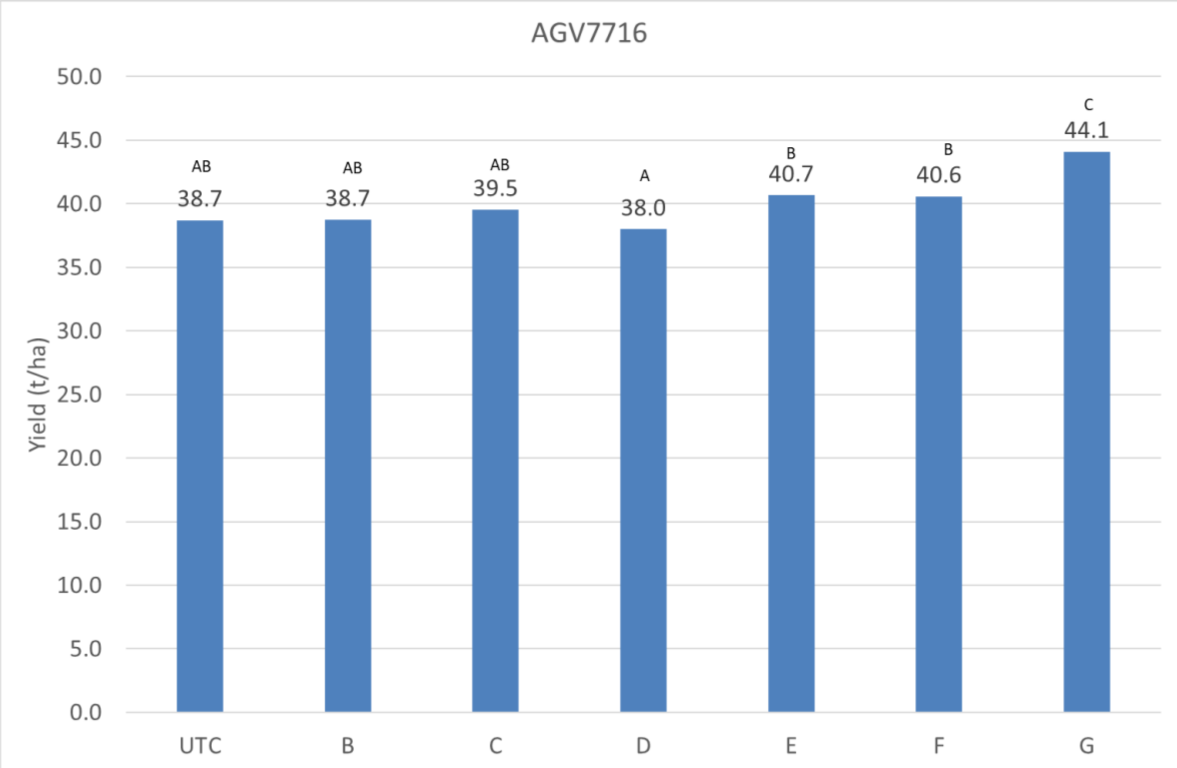

Figure 6.2 Yield (t/ha) as a result of the various spray treatments. Values above columns followed by the same character are not significantly different (P=0.05).

Conclusion

No phytotoxicity was observed, the biological crop protection products used were crop safe.
Based on the StAUDPC treatments B, C, D (Humic acid treatments) and E, F showed no efficacy to control potato late blight, disease severity was comparable to the untreated control. Treatment G did show a suppressing effect on the development of potato late blight.

7 Conclusions and recommendations

Based on the literature research the interviews of experts and the experiments, it can be concluded that:

- There are several mechanisms of humic acids described that might stimulate plant growth. Depending on the situation the effect of humic acids can be high or low. On average, yield increases can be expected. In the Netherlands optimal growing conditions might be the reason that positive effects of humic acids on plant growth under field conditions are not observed. The field experiments described in this report confirm this.
- Humic acids of Ecoson is intended as a plant biostimulant, not a food/feed additive because of the animal manure status.
- Humic acids of Ecoson can be sprayed with normal field sprayers in doses 120 l Humic acids of Ecoson/hectare. Crop spraying with doses 1,5-3 l Humic acids of Ecoson/hectare per spraying (two times spraying) seemed safe for onion, potatoes and tomato plants.
- In experimental setups application rates are within the range 1.5 to 20 kg humic substances/ha. Optimal application rates depend on humic substances product and crop. Application rates of circa 8 kg humic substances/ha are thought to be an adequate guideline.
- A lot of research about Humic acids is done but little information is found on the use of humic acids under field situations in the Netherlands.
- Humic acids are used for fruit trees for stimulating root growth and for their chelating properties. This might be an interesting market for humic acids.
- Cultivations under sub optimal conditions might be markets for humic acids (for instance organic farmers).

Marketing of humic acids requires further proof of product. Field experiments are needed followed by word-of-mouth advertising. Not only the farmers should be persuaded but also the advisors or fertilizer suppliers.

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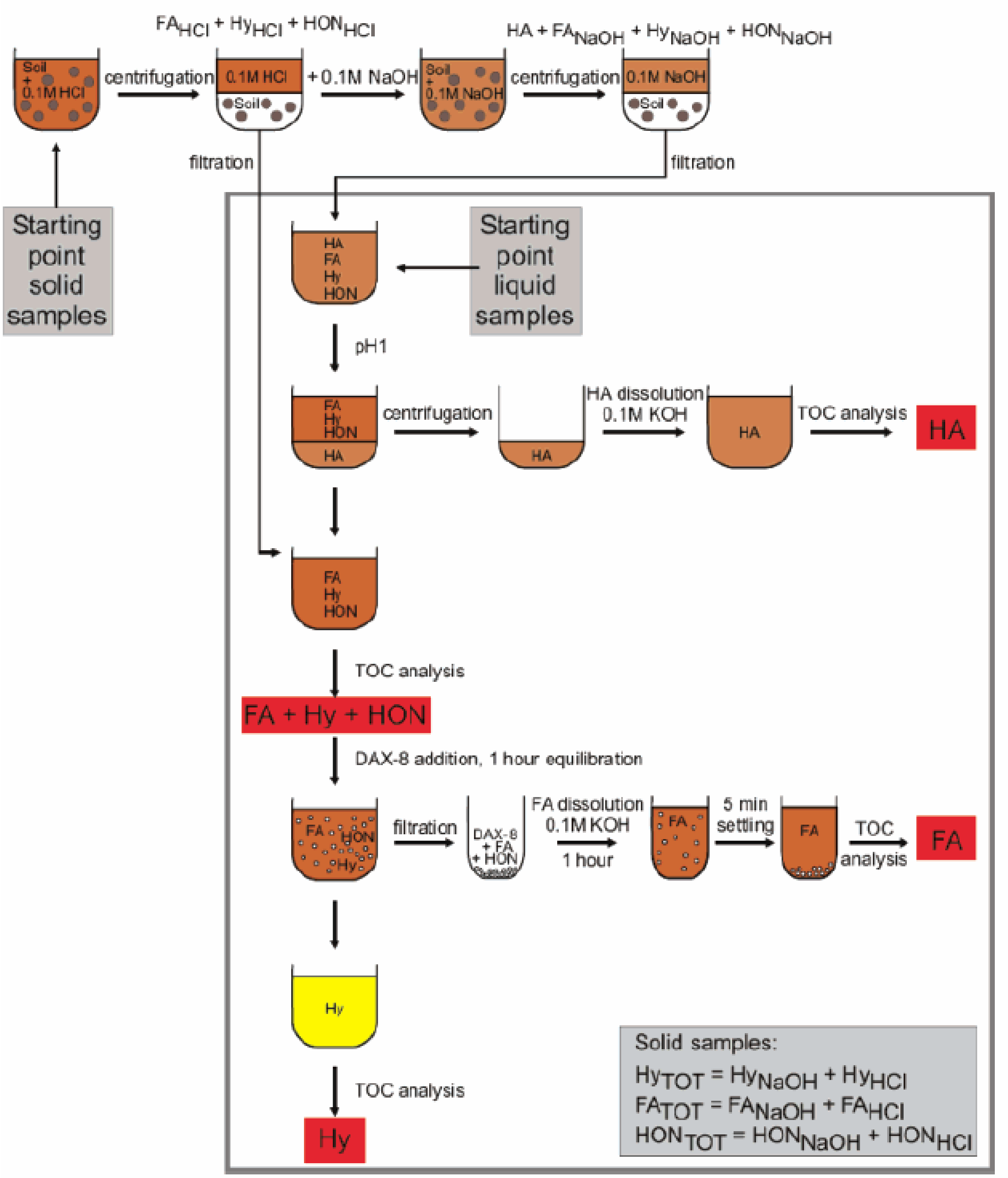
Appendix 1 Scheme for extraction of humic acids



Appendix 2 Table A2.1 Summary of research on humic acid products. Source: Calvo et al. (2014)

Plant Soil (2014) 383:3–41

Table 1 Summary of reported effects of humic substances on plant growth, nutrient uptake, and plant physiology

Crop	Type of Humic Substance	Reference	Study Conditions	Reported Effects on Growth and Nutrient Uptake	Effects on Plant Physiology
Cucumber (*Cucumis sativus*)	Humic acid	Aguirre et al. 2009	Growth chamber	No effect on root growth	Increased transcription of genes encoding Fe(III) chelate-reductase (CsFRO1) and an Fe(II) root transporter (CsIRT1); increase H^+-ATPase activity
Cucumber	Humic acid	El-Nemr et al. 2012	Field tests in two years with foliar sprays	Increased plant growth and yield; enhanced uptake of N, P, K, Ca, and Mg	
Cucumber	Humic acid	Karakurt et al. 2009	Yield and fruit- quality study in ground in organic production greenhouse conducted in two years	Increased total fruit yield, total soluble sugars, reducing sugars, and chlorophyll b	
Cucumber	Humic acid	Mora et al. 2010	Hydroponic culture in growth chamber	Increased shoot growth; increased NO_3 in shoots and decreased NO_3 in roots	Increased H^+-ATPase activity and significant changes in root-to-shoot distribution of NO_3, cytokinins, and polyamines.
Cucumber	Fulvic acid	Rauthan and Schnitzer 1981	Growth chamber hydroponic culture	Increased shoot and root dry weight, numbers of flowers per plant, and uptake of N, P, K, Ca, Mg, Cu, Fe, and Zn	
Multiple, including vegetables, tomato, cereals, ornamentals and grape (*Vitis vinifera*)	Humic substance	Morard et al. 2011	Hydroponic culture and field trial (grape)	With some of the tested plants, increased plant fresh weight, number of flowers, and water use efficiency. With grape, increased N content of must	
Grape (*Vitis vinifera*)	Humic substance	Sánchez-Sánchez et al. 2006	Field trials over two years testing combination of Fe chelates with humic substances	Increased uptake of P and Fe; decreased uptake of Na	
Micro-Tom tomato (*Solanum lycopersicum*)	Humic substances	Canellas et al. 2011	Germination paper in growth chamber	Enhanced number of lateral roots	Auxin-like activity detected by activation of the auxin synthetic reporter DR5: GUS
Arabidopsis thaliana, tomato, maize (*Zea mays*)	Humic substances	Dobbss et al. 2010	Growth chamber	Increased lateral root emergence	Increased H^+-ATPase activity in root vesicles, activated auxin synthetic reporter DR5: GUS
Arabidopsis thaliana, micro-Tom tomato	Fulvic acid	Dobbss et al. 2007	For Arabidopsis, mini-hydroponic system in growth chamber.	Increased lateral root growth in Arabidopsis and wild-type micro-Tom tomato.	No promotion of lateral root emergence with dgt tomato mutant insensitive to IAA
Tomato	Humic acids	Adani et al. 1998	Hydroponic culture	Increased growth of roots and shoots; enhanced uptake of N, P, Fe, and Cu	
Tomato	Humic acid	Yildrim 2007	In-ground greenhouse test for yield conducted during two growing seasons	Increased early and total yield in both years; increased total soluble solids and ascorbic acid content in fruit	
Tomato	Fulvic acid and humic acid	Lulakis and Petsas 1995	Growth chamber tests with seedlings in Petri plates	Enhanced root and shoot growth at 14 days after seeding	
	Fulvic acid and humic acid	Chen et al. 2004			

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13

Table 1 (continued)

Crop	Type of Humic Substance	Reference	Study Conditions	Reported Effects on Growth and Nutrient Uptake	Effects on Plant Physiology
Soybean (Glycine max), melon (Cucumis melo), and ryegrass (Lolium perenne)			Hydroponic culture in growth chambers with differing levels of Fe and Zn	Increased SPAD readings (chlorophyll measure) in all three plant systems with fulvic acid and humic acid	
Basil (Ocimum basilicum)	Humic acid with and without PGPR	Befrozfar et al. 2013	Field tests with seed treatments and foliar sprays	Increased yield of oil with humic acid alone and in combination with PGPR	
Okra (Abelmoschus esculentus)	Humic acids	Kim et al. 2010	In-ground test inside wire house	Increased yield (fruits per plant) at recommended fertility but not at 50 % fertility level	
Potato (Solanum tuberosum)	Humic acid	Selim et al. 2012	Field study with different water regimes; application through fertigation system	Enhanced tuber yield; increased percent protein and ascorbic acid content in tubers; increased SPAD readings (chlorophyll indicator) in leaves	
Wheat (Triticum aestivum)	Humic acid	Tahir et al. 2011	Pot trial with calcareous and non-calcareous field soils with three levels of N, P, and K	Increased plant height and dry weight of roots and shoots; enhanced uptake of N	
Wheat	Fulvic acid	Dunstone et al. 1988	Glasshouse, growth chamber, and field trials with foliar sprays of fulvic acid	Increased plant growth in some studies but not in others. No increases in yield or water use in field tests	Decreased stomatal conductance in many studies but no relation to plant growth response
Wheat	Fulvic acid	Xudan 1986	Pot and field experiments with foliar sprays of fulvic acid	Enhanced chlorophyll content; increased roots uptake of ^{32}P; partial alleviation of grain yield depression by moderate drought	Decreased stomatal conductance
Wheat	Fulvic acid	Peng et al. 2001	Hydroponics with varying levels of Se as sodium selenite	Enhanced seedling root growth with low levels of Se, reduced symptoms of Se toxicity with high levels of Se	Reduction in Se-induced cell membrane permeability and free-proline content with fulvic acid
Wheat	Fulvic acid	Gu et al. 2001	Hydroponics with 8 concentrations of 3 rare earth elements (La^{3+}, GD^{3+}, and Y^{3+})	Increased bioaccumulation of La^{3+}, GD^{3+}, and Y^{3+} in roots and shoots, resulting in less buildup in soil	Activation of glutamic oxaloacetic transaminase (GOT) enzyme
Maize (Zea mays)	Humic acids	Jindo et al. 2012	Lab assays in minimal liquid medium	Promotion of root growth; increased number of mitotic sites on roots	Increased number of mitotic sites and proton pump activity in roots
Maize	Humic substances	Schiavon et al. 2010	Growth chamber test in hydroponics	Not assessed	Enhancement of phenylpropanoid pathway, decrease in phenylalanine and tyrosine, increase in phenolic compounds and some amino acids
Maize	Humic acids	Canellas et al. 2002	Lab assays in minimal liquid medium	Increased root elongation, proliferation of secondary roots, and root surface area	Simulated H^+-ATPase activity of plasma membrane and mitotic sites of lateral root development
Maize	Fulvic acid	Anjum et al. 2011b	Pot trials in net house under drought and no drought conditions	Increased leaf area, plant dry weight, chlorophyll content, and yield under drought stress; increased yield under non-drought conditions	Increased assimilation rate of CO_2 and content of proline
Maize	Fulvic and humic acids	Eyheraguibel et al. 2008			

Table 1 (continued)

Crop	Type of Humic Substance	Reference	Study Conditions	Reported Effects on Growth and Nutrient Uptake	Effects on Plant Physiology
			Application to 10-day old seedlings with growth in hydroponic culture until cob filling stage	Increased root length of seedlings; increased total plant biomass at 2 months; enhanced plant development as noted with increased numbers of leaves and flowers per plant; increased lateral root development; increased nutrient uptake	
Maize	Humic acids	Canellas et al. 2009	Lab assays in minimal liquid medium	Stimulated root growth and root length	Activated proton pump activation in root plasma membrane vesicles
Maize	Humic acids	Asli and Neumann 2010	Growth chamber study in hydroponic culture and in soil with multiple applications	Inhibition of shoot but not root growth with high concentrations of humic acid; reduced transpiration	Reduced hydraulic conductivity reduced water transport from root medium to shoot
Maize	Fulvic acid and humic acid	Harper et al. 1995	Seedling growth for four days in nutrient solution with and without aluminum	Enhanced root elongation in absence of Al; in presence of Al, alleviated Al toxicity limitation of root elongation	
Pear (Pyrus communis)	Humic acid	Marino et al. 2010	In vitro micropropagation conditions of shoot cultures	Improved acclimatization and micropropagation; increased rooting, plant height, chlorophyll content, and nutrient uptake	Reduced activity of catalase and malondialdehyde
Pepper (Capsicum annuum)	Humic acid	Cimrin et al. 2010	Growth chamber in soil mix	Increased shoot and root weights, also increased N, P, K, Ca, Mg, S, Mn, and Cu under moderate salt stress conditions	Suggested that supramolecular agglomerates of humic acid limit root water transport, resulting in restricted shoot growth
Pepper	Humic acids	Karakurt et al. 2009	In-ground greenhouse test for yield conducted during two growing seasons	Increased total yield, early yield, mean fruit weight, total soluble sugars, and chlorophyll b	
Pepper	Fulvic acid	Aminifard et al. 2012	Field trial with drenches of fulvic acid during vegetative growth	Not recorded	Increased fruit content of carbohydrate, total phenolics, capsaicin, and carotenoids; increased antioxidant activity in fruit
Lantana camara	Humic acid and fulvic acid	Costa et al. 2008	Greenhouse test in soilless mix for propagation	Increase biomass of roots and shoots, earlier flowering of rooted cuttings	Upregulation of AGAMOUS-like gene (AGL)
Lemon trees (Citrus limon) on C. macrophila rootstock	Fulvic acid	Sánchez-Sánchez et al. 2002	Field test in orchard with calcareous soil	Increased foliar uptake of Fe and Cu; increased yield (fruit weight), fruit equatorial diameter, juice pH, and vitamin C	
Wild olive (Olea europaea)	Fulvic acid	Murillo et al. 2005	Field tests in soils polluted by trace elements under semi-arid conditions	Increased N and chlorophyll content in plants without increases to phytotoxic levels of Cd, Cu, Pb, Tl, or Zn	
Greek fir (Abies cephalonica)	Fulvic acid	Zancani et al. 2011	Study with embryonic cell lines to evaluate the hormone-like effects		Fulvic acid interacted with the plant hormonal signaling pathway;

Table 1 (continued)

Crop	Type of Humic Substance	Reference	Study Conditions	Reported Effects on Growth and Nutrient Uptake	Effects on Plant Physiology
			of fulvic acid on stages of somatic embryogenesis	Increased proliferation rate and percentage of pro-embryonic masses	increased cellular ATP and glucose-6-phosphate
Beech (Fagus sylvatica)	Fulvic acid	Asp and Berggren 1990	Growth chamber and greenhouse tests with seedlings	Fulvic acid-complexed Al was not taken up by roots; reduced root uptake of Al and ^{32}P-phosphate	
Sunflower (Helianthus annuus)	Fulvic acid	Bocanegra et al. 2006	Growth chamber test with seedlings in Hoagland solution with Fe provided in dialysis bag	Increased release and mobilization of Fe from iron chelates; increased plant uptake of the released Fe; concluded that fulvic acid chelated Fe^{3+} for plant uptake	
Rice (Oryza sativa)	Fulvic acid	Pandeya et al. 1998	Growth chamber test with seedlings in calcareous soil with Fe-fulvic acid, $FeCl_3$ and ^{59}Fe tracer	Fe uptake was greater with application of Fe-fulvic acid than $FeCl_3$	Suggested that fulvic acid could overcome the rate-limiting step of transporting Fe from the soil solution to plant roots by diffusion
Rice	Humic acid	García et al. 2012	Growth chamber test in nutrient solution and water stress via evaporation	Increased plant growth and biomass under water deficit conditions; reduced oxidative stress of plants under water stress	Induced peroxidases leading to reduced H_2O_2 content and maintenance of membrane permeability; increased proline content of plants
Rice	Humic acid	García et al. 2013	Growth chamber test in nutrient solution and water stress induced by polyethylene glycol	Under water stress, humic acid maintained peroxidase activity below levels in plants without humic acid; lipid peroxidation was lower in water-stressed plants with HA than in stressed plants without HA; abscisic acid (ABA) levels were similar in stressed plants with and without HA.	Suggested that protection against water stress resulted from ABA-independent mechanisms involving regulation of tonoplast aquaporin genes (OsTIPs)
Common bean (Phaseolus vulgaris)	Fulvic acid	Poapst and Schnitzer 1971	Test with hypocotyl sections of bean seedlings	Increased number of adventitious roots with fulvic acid with and without IAA	
Common Bean	Humic acid	Aydin et al. 2012	Greenhouse test evaluating humic acid for mitigation of salinity stress	Reduced plant death with humic acid treatments at high doses of NaCl, $CaCl_2$, $MgCl_2$, and KCl; increased plant root and shoot dry weight; increased nitrate content	Under salinity stress, humic acid increased proline and electrolyte leakage of plants
Broad bean (Vicia faba)	Fulvic acid	Shahid et al. 2012	Growth chamber test in modified Hoagland solution	At low concentrations, fulvic acids complexed toxic free Pb^{2+} and increased Pb uptake without causing Pb toxicity; at high concentrations, FAs reduced Pb uptake and toxicity	Pb without fulvic acid induced H_2O_2 and lipid peroxidation; fulvic acid delayed lipid peroxidation
Chrysanthemum (Chrysanthemum indicum)	Humic acid	Mazhar et al. 2012	In ground greenhouse test conducted over two seasons with salinity stress	Increased vegetative growth, flowering, total carbohydrates, N,	

Table 1 (continued)

Crop	Type of Humic Substance	Reference	Study Conditions	Reported Effects on Growth and Nutrient Uptake	Effects on Plant Physiology
				P, and K with humic acid under salinity stress	
Pistachio (Pistacia vera)	Humic acid	Moghaddam and Soleimani 2012	Test of humic acid for mitigating salinity stress	Increased shoot growth with humic some acid treatments under salinity stress	Decreased levels of abscisic acid and proline with some humic acid treatments

Appendix 3 Analytical report Koch Eurolab

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LABORATORIUMANALYSES

RAPPORTNUMMER: 191298702

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RAPPORT: 191298702

Wageningen Plant Research
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ORGANISCHE MESTSTOF ANALYSE: HUMUSZUREN en FULVINEZUREN

Uw monsteraanduiding	Labnummer	MONSTER DATUM
101 Humus Ecosun	98702	03-12-2019

Parameters	Resultaat	Eenheid
Humuszuren	**10.5**	g /kg product
Fulvinezuren	**2.4**	g /kg product

monstermateriaal

Toelichting:
Klassieke humuszuren analyse: schudverhouding 1 op 50, extractant 0.25M KOH eerste extract humuszuren + fulvinezuren, daarna afscheiding van fulfinezuren door toevoegen zoutzuur tot pH 2. Daarna sprectrofotometrische analyse in vergelijking met een standaard met opgeloste humuszuren.

Rapportnummer: 191298702 8-Jan-2020

Layoutnr.: 4 juli 2017 3HUM.XLTX

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RAPPORT 191298702

Wageningen Plant Research
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ORGANISCHE MESTSTOF ANALYSE OP BEMESTENDE WAARDE EN ZWARE METALEN

Datum ontvangst	3-dec-2019	Labnr. 98702
Rapportagedatum	8-jan-2020	
Partij-aanduiding	101 Humus Ecosun (soort monster)	
Producent	Wageningen Plant Research	

PARAMETER	**ANALYSE RESULTAAT**		
BEMESTENDE WAARDE IN kg per TON PRODUCT (= gram per KILO)			
Droge stof	**122**		
Vocht	**878**		
Organische stof	**70.4**		
Ruw as	**52**		
pH	**8.36**		
Totaal stikstof (N)	**12.4**		
Fosfaat (gerekend als P_2O_5)	**2.0**		
Kali (gerekend als K_2O)	**9.4**		
Calcium (gerekend als CaO)	**0.4**		
Magnesium (gerekend als MgO)	**0.03**		
Natrium (gerekend als Na_2O)	**3.4**		
Nitraat (gerekend als N)	**< 0.01**		
Ammonium (gerekend als N)	**0.04**		
Zwavel totaal (gerekend als S)	**4.2**		
		norm compost *)	beoordeling
Organische stof (gew % in de droge stof)	**57.7**	>10	voldoet
ZWARE METALEN IN mg PER kg DROGESTOF			
Cadmium (Cd)	**1.0**	≤ 1	voldoet
Chroom (Cr)	**16**	≤ 50	voldoet
Koper (Cu)	**605**	≤ 60	te hoog
Kwik (Hg)	**0.3**	≤ 0.3	voldoet
Nikkel (Ni)	**110**	≤ 20	te hoog
Lood (Pb)	**4**	≤ 100	voldoet
Zink (Zn)	**1762**	≤ 200	te hoog

Het monster voldoet niet aan de wettelijke eisen voor compost.

Met product wordt bedoeld het monster zoals ontvangen, ongedroogd. Dit rapport is bedoeld om een productieproces te beoordelen en advies, maar niet voor RVO doeleinden.

Dhr. C.F.M. Koch (directeur)

Rapportnummer: 191298702
Layoutnr.: 28 aug 2018 7ZBC.XLT

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RAPPORT: 191298702

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ORGANISCHE MESTSTOF ANALYSE: HUMUSZUREN en FULVINEZUREN

Uw monsteraanduiding	Labnummer	MONSTER DATUM
102 Humus x	98703	03-12-2019

Parameters	Resultaat	Eenheid
Humuszuren	6	g /kg product
Fulvinezuren	13.9	g /kg product

monstermateriaal

Toelichting:
Klassieke humuszuren analyse: schudverhouding 1 op 50, extractant 0.25M KOH eerste extract humuszuren + fulvinezuren, daarna afscheiding van fulfinezuren door toevoegen zoutzuur tot pH 2. Daarna sprectrofotometrische analyse in vergelijking met een standaard met opgeloste humuszuren.

Rapportnummer: 191298702 8-Jan-2020

Layoutnr.: 4 juli 2017 3HUM.XLTX

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RAPPORT 191298702

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ORGANISCHE MESTSTOF ANALYSE OP BEMESTENDE WAARDE EN ZWARE METALEN

Datum ontvangst	3-dec-2019	Labnr. 98703
Rapportagedatum	8-jan-2020	
Partij-aanduiding	102 Humus x (soort monster)	
Producent	Wageningen Plant Research	

PARAMETER	**ANALYSE RESULTAAT**	norm compost *)	beoordeling
BEMESTENDE WAARDE IN kg per TON PRODUCT (= gram per KILO)			
Droge stof	**290**		
Vocht	**710**		
Organische stof	**137**		
Ruw as	**153**		
pH	**6.83**		
Totaal stikstof (N)	**1.4**		
Fosfaat (gerekend als P_2O_5)	**31.2**		
Kali (gerekend als K_2O)	**50.3**		
Calcium (gerekend als CaO)	**0.7**		
Magnesium (gerekend als MgO)	**1.1**		
Natrium (gerekend als Na_2O)	**16.0**		
Nitraat (gerekend als N)	**< 0.01**		
Ammonium (gerekend als N)	**0.06**		
Zwavel totaal (gerekend als S)	**9.7**		
Organische stof (gew % in de droge stof)	**47.2**	>10	voldoet
ZWARE METALEN IN mg PER kg DROGESTOF			
Cadmium (Cd)	**0.1**	≤ 1	voldoet
Chroom (Cr)	**2.2**	≤ 50	voldoet
Koper (Cu)	**6.9**	≤ 60	voldoet
Kwik (Hg)	**0.1**	≤ 0.3	voldoet
Nikkel (Ni)	**6.6**	≤ 20	voldoet
Lood (Pb)	**1.7**	≤ 100	voldoet
Zink (Zn)	**22**	≤ 200	voldoet

Het monster voldoet aan de wettelijke eisen voor compost.

Met product wordt bedoeld het monster zoals ontvangen, ongedroogd. Dit rapport is bedoeld om een productieproces te beoordelen en advies, maar niet voor RVO doeleinden.

Dhr. C.F.M. Koch (directeur)

Rapportnummer: 191298702

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Appendix 4 Description of potato experiment

Material and methods

In 2018, an experiment with potatoes and addition of humic acid product was conducted at a potato field of 1 hectare located between the Buntstraat and Koeveringsdijk in St. Oedenrode. Before the potatoes were planted, four alternate beds were treated with humic acid product and four beds were left untreated. All other treatments for cultivation (fertilisation, pesticides) were the same. The soil type was a sandy soil. The potato cultivar Hansa was used. The experiment started in May 2018 and ended in October 2018.

A dose of 120 L per hectare of the humic acid product of Ecoson was mixed with 80 L water, after which the product was applied to the soil. The location of the treated and non-treated beds was recorded with GPS.

The growth of tubers during the growing season was determined by PEKA KROEF with a protocol they use to determine yield per hectare.

Results

Application of the humic acid product to the field was easy and no problems like clogging appeared. The climate conditions were not in favour of the growth of potatoes, especially in cultivation on sandy soils, as there was too little water available. Therefore growth of tubers was slow and the farmer and PEKA KROEF decided that sampling before harvest was not needed. Visually, there was no difference between development of tubers with or without humic acid product treatment.

Conclusions

The research conducted was very basic.

It was concluded that:

- Humic acid product of Ecoson can be applied with normal agricultural machinery

In 2018, adding the humic acid product did not lead to differences in potato tuber growth, but this was only determined by a quick visual inspection.

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

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