2019.02.20
Version 4
689229

Bioproduct Valorisation

D4.9 - Field test report Season 2018

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 689229.

DECISIVE

A DECENTRALISED MANAGEMENT SCHEME FOR INNOVATIVE VALORISATION OF URBAN BIOWASTE

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 689229.

A Decentralised Management Scheme for Innovative Valorisation of Urban Biowaste

D4.9 – Field Test Report

Grant Agreement N°	6689229		
Acronym	DECISIVE		
Full Title	A Decentralised Management Scheme for Innovative Valorisation of Urban Biowaste		
Work Package (WP)	WP4		
Authors	DUVAL Bérengère, JUGE Chloé		
Document Type	Deliverable		
Document Title	D4.9 Field test report		
Dissemination Level	**CO**	Confidential, only for partners of the Consortium (including the Commission's Services)	
	PU	Public	**X**
	PP	Restricted to other programme participants (including the Commission Services)	
	RE	Restricted to a group specified by the Consortium (including the Commission Services)	

ABSTRACT

This report analyses the potential of biofertilizers and biopesticides produced from anaerobic digestion and solid-state fermentation of urban biowaste to be used in urban farming. Three field tests were run in Season 2018. The first one focused on liquid bioproducts fertilizing potential in hydroponic system. In this test a selection of the best commercial mineral fertilizer and commercial organic fertilizer was made and the best formulation for digestate was studied. Those preliminary trials were made in order to prepare an efficient comparison in Season 2019. The second test explored solid bioproduct fertilizing potential in horticultural container system. The third test studied the biopesticide potential of *Bacillus thuringiensis* developed using solid state fermentation on *Pieris brassicae* pest. This report presents the Season 2018 results for the three field tests and the outlooks for Season 2019.

DOCUMENT HISTORY

version	date	editors	modification
Version 1	30/06/18	Bérengère Duval	First draft
Version 2	07/01/19	Bérengère Duval	Second draft for approval by the reviewers
Version 3	11/02/2019	Bérengère Duval	Third draft for approval by the reviewers
Version 4	20/02/2019	Bérengère Duval	Fourth version for submission

CONTRIBUTORS

name	company	contributions include
DUVAL BÉRENGÈRE	**REFARMERS**	**AUTHOR**
JUGE CHLOÉ	**REFARMERS**	**CO-AUTHOR**
FAYOLLE ALEXIS	**REFARMERS**	**FIELD TESTS**

REVIEWERS

name	company	contributions include
CERDA LLANOS ALEJANDRA	**UAB**	**REVIEW**
CHIFARI ROSARIA	**ENT**	**REVIEW**
DEGUEURCE AXELLE	**IRSTEA**	**REVIEW**
FAYOLLE ALEXIS	**REFARMERS**	**REVIEW**
MEJIAS LAURA	**AERIS**	**REVIEW**
REINO CLARA	**AERIS**	**REVIEW**
RODRIGUEZ SERRANO PAULA	**UAB**	**REVIEW**
SCOTTI MARCO	**GEOMAR**	**REVIEW**

To ease the reading of this report, we have separated it into three sub-reports: Liquid fertilizers, Solid fertilizers and Biopesticide

Table of contents

Table of figures

Table of tables

Glossary

AD:	Anaerobic Digestion
ADEME:	Agency of the environment and the control of energy (Agence de l'environnement et de la maitrise de l'énergie - France)
AMF:	Arbuscular Mycorrhizal fungus
B:	Block
Bt:	*Bacillus thuringiensis*
CT:	Compost tea
CFU:	Colony Forming Unit
E:	Edge line
EBIC:	European Biostimulants Industry Council
EC:	Electroconductivity
F:	Fungal product
FAO:	Food and Agriculture Organization of the United Nations
GHE:	General Hydroponics Europe
HA:	Humic acid
HP:	Hydroponic system
IBMA:	International Biocontrol Manufacturers Association
IRSTEA:	Research Institute of Science and Technology for Environment and Agriculture (Institut national de recherche en science et technologie pour l'environnement et l'agriculture - France)
K:	Potassium
L:	Line
L-digestate:	Liquid phase extracted from the anaerobic digestion of urban biowaste
L-SSF:	Liquid phase extracted from the solid-state fermentation of solid digestate
mAD:	micro Anaerobic Digester
M:	Molasses
N:	Nitrogen
P:	Potassium
Pm:	Product mineral
Po:	Product organic
S-digestate:	Solid phase extracted from the anaerobic digestion of urban biowaste
S-SSF:	Solid phase extracted from the solid-state fermentation of solid digestate
SSF:	Solid State Fermentation
T4P:	Pilot Project of Parisian Productive Rooftop
UAB:	Autonomous University of Barcelona (Universitat Autònoma de Barcelona - Spain)
ZG:	ZipGrow™

General introduction and context

DECISIVE PROJECT

DECISIVE principle

DECISIVE project proposes to valorise urban biowaste using a decentralized management scheme. The objective is to promote the use of biowaste produced in cities as raw material to develop heat, energy, biofertilizers and biopesticides to use locally and in urban farming. Therefore, the project contributes to shift from a linear paradigm to a circular concept, by closing the organic loop. This concept is tested on two pilot sites: in Lyon (France) and in Barcelona (Spain). The present report is a deliverable from DECISIVE project.

The four bioproducts studied

Figure 1 shows that the pilot includes two technologies: a micro Anaerobic Digester (mAD) and a solid-state fermentation (SSF) reactor. Besides biogas, mAD produces digestate that is separated in two phases: a solid phase that is called in this report solid digestate (S-digestate) and a liquid phase that is called liquid digestate (L-digestate). They both have potential as fertilizers. Part of solid digestate is used in SSF reactor with the aim of producing biopesticides, using *Bacillus thuringiensis* (*Bt*) as inoculum (gram-positive bacteria used as biopesticide in organic farming). Solid product from SSF is called here solid SSF (S-SSF). A liquid product called liquid SSF (L-SSF) is extracted from S-SSF. Both have potential as fertilizers and biopesticide.

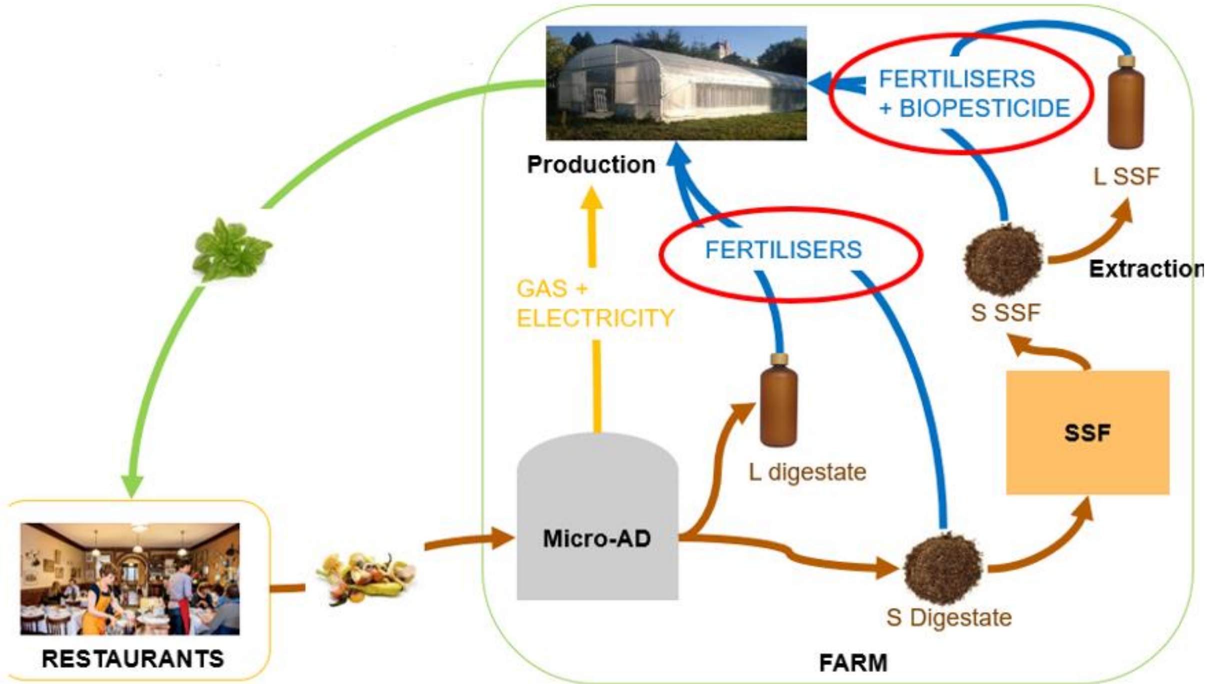


Figure 1: Lyon pilot for DECISIVE scheme

URBAN BIOWASTE POTENTIAL

Cities occupy 2 % of the world's land surface but use 75 % of its resources and release similar proportions of waste (Girardet, 2000). In Europe, 280-300 kg/year of food are lost and wasted per capita, in which 95-115 kg/year per capita are wasted by consumers (FAO, 2011). The annual quantity of food waste generated in the European Union is estimated at 89 million tons and it is expected to increase to 126 million tons by 2020 in absence of additional prevention policy or activities (European Commission, 2010). Considering that around one third of all food waste is edible (WRAP *et al.*, 2009), there are still two thirds that need to be valorised. Developing biorefineries applying organic fraction of municipal solid waste as feedstock presents a promising opportunity for moving up the waste hierarch by coupling the waste and production sector in a future circular bioeconomy (Vea *et al.*, 2018). Anaerobic digestion (AD) and solid-state fermentation (SSF) appear as particularly interesting ways to valorise urban biowaste.

ANAEROBIC DIGESTION

Definition

AD is a technology based on the anaerobic degradation of organic matter by microorganisms, in controlled conditions. This degradation produces a digestate, which is an organic matter-rich humid product, and a biogas composed at 50 to 70 % of methane (CH_4), 20 to 50 % of carbon dioxide (CO_2) and some trace gases (NH_3, N_2, H_2S). Waste treated by AD can be farm and agribusiness effluent, organic waste, green waste, biowaste from industry or household, and sewage sludge (ADEME, 2018).

Uses and benefices for the biogas and digestate

The biogas can be used to produce energy. As it is methane rich, it represents an interesting fuel. It is mainly valorised by combustion in an engine to produce electricity or heat. After elimination of the carbon dioxide, it can also be used as fuel or injected in the natural gas network. The digestate, liquid or solid, can be used as a fertilizer in the agricultural sector. It is spread on agricultural land instead of using organic amendment or liquid fertilizers (ADEME, 2018). The AD has the advantage of reducing the volume of waste as well as the emission of greenhouse gases by reducing the use of fossil energy and chemical fertilizers. It gives the possibility to valorise greasy organic waste not compostable and to decrease the emissions of odours with closed equipment (Ministère de la transition écologique et solidaire, 2018). Greasy organic waste could be valorised by compost if mixed with other substrates, however AD reduces the valorization process time compared to composting. Besides, AD presents a very positive energy balance due to the production of methane compared to composting technology. Appropriate for humid and highly biodegradable waste, AD could be an alternative to composting technology (ADEME, 2015).

SSF PROCESS

Definition

Solid-state fermentation (SSF) is defined as a process that takes place in a solid matrix in absence or near absence of free water (Thomas *et al*., 2013). SSF is an attractive technology that allows the use of solid substrates for biotransformation into value-added bioproducts (Cerda *et al*., 2016). Hydrolytic enzymes (El-Bakry *et al*., 2015), biosurfactants (Jimenez-Penalver *et al*., 2016; Vishal and Aniruddha, 2012), aromas (Martinez *et al*., 2017), biopesticides (Ballardo *et al*., 2016) or bioplastics (Castilho *et al.*, 2009) have been successfully produced by SSF using different organic waste.

Digestate as feedstock for biopesticide production

SSF can also be performed using digestate as feedstock. In particular, the Universitat Autònoma de Barcelona (UAB), partner of the DECISIVE project, is studying the use of digestate through SSF to produce a biopesticide based on the microorganism *Bacillus thuringiensis* (*Bt*). UAB has especially been working on scaling-up the process of SSF with digestate as the feedstock (the previously mentioned studies were mostly at lab scale). Digestate has been subjected to hygienisation process according to EU regulation. Thus, a large amount of biopesticide material can be produced by the SSF reactor.

URBAN AGRICULTURE

Concept

There is no strict definition of urban farming, but a commonly recognized one is the following: “Urban agriculture is generally characterized by closeness to markets, high competition land, limited space, use of urban resources such as organic solid waste and wastewater, low degree of farmer organization, mainly perishable products, high degree of specialization” (Veenhuizn and Van, 2006). Urban agriculture is not only defined by being urban. It is also part of the local economy, social and ecological systems (Mougeot, 2000). It includes both non-profit system with inhabitant in sharing garden, and profit system with professional growers who sell their production (Daniel, 2013).

Evolution

Urban population is increasing all over the world, putting enormous pression on cities infrastructure and resources. (Véron, 2007). As one of the solutions to increase cities resilience, urban farming has strongly developed over the past decades, especially in the Northern Hemisphere (Girard *et al*, 2015). Urban farming is already well developed as commercial system in North America cities like Montreal, New York or Toronto (Thomaier, 2014; Specht *et al*, 2013).

Different systems

Urban farming usually grows in containers, in hydroponic systems, aquaponic systems or in soil grown systems. Hydroponic systems are defined as the production of plants in nutritive solutions, without natural soil (Larousse, 2018). Aquaponic systems are defined as hydroponics combined with fish farming. Fish waste is turned into nutrients, following the nitrification cycle. The nutrients can be assimilated by plants, creating a quasi-autonomous ecosystem. Hydroponic and aquaponic systems are particularly interesting as light systems to install on cities rooftops. Urban agriculture can also occur in horticultural containers, using a growing substrate. This form of urban farming is often used in shared community garden. Urban farming can be implemented on rooftops, parking, and warehouses, unused urban or peri-urban land.

FIELD TESTS OBJECTIVES

Three field tests were run on the four bioproducts in greenhouses and were season dependent. This document presents Season 2018 results of the field tests. “Season” term was chosen over “Year” term as the trials could only be run between April and December because of climate conditions.

“Liquid fertilizers” field test ran preliminary hydroponics trials in order to identify the best mineral and organic fertilizers to compare to L-digestate and L-SSF in Season 2019. It studied as well the best formulation and protocol for using those two bioproducts as fertilizers in hydroponics.

“Solid fertilizers” test studied the fertilizing potential of S-digestate in containers according to the structure and composition used and compared to compost.

The last test, denoted as “Biopesticide”, explored the biopesticide potential of L-SSF when sprayed compared to a commercial solution that was also based on *Bt*.

During Season 2019, the potential of the bioproducts will be investigated further and they will be compared to commercial products. In Season 2018, the Lyon pilot AD plant was not installed and bioproducts were provided by partners of the project. Season 2019 will aim to use bioproducts from Lyon and Barcelona pilot plants.

To ease the reading and understanding, each field test within this report is presented in a different section organized as an individual report with its own Table of contents. In each section, chapter 1 describes the specific state-of-the-art, chapter 2 explains the objectives and chapter 3 focuses on the materials and methods. All sections end with chapter 4 that presents and discusses the results obtained and chapter 5 that proposes conclusions and presents the outlook for Season 2019. A global conclusion for Season 2018 is presented at the end of the three sections.

General state-of-the-art

Below cross-subjects concerning state-of-the-art on fertilization are detailed: sections "Liquid fertilizers" and "Solid fertilizers". Specific subjects are detailed in each section's state-of-the-art.

FERTILIZATION MANAGEMENT

In agriculture, fertilizing matters are "products aimed to assure or increase the nutrition of plants and physical, chemical and biological properties of the soil". Fertilizers include manure, amendment, and matter stimulating natural process of plants and soil (Code rural et de la pêche maritime - France, 2018).

DIGESTATE, PRODUCT OF ANAEROBIC DIGESTION

Fertilizing power

The AD degrades between 20 and 95 % of the feedstock organic matter, depending on its composition. The unconsumed matter, named digestate, can be used as a fertilizer for crops in agriculture. The digestate can be spread directly on the ground, as manure, or treated first and then spread. According to the actual legislation digestate needs to be hygienized or composted to be spread without any problem especially if it is coming from the AD of organic urban waste. Digestate has not yet the label of end of waste as it is. End of waste for digestate is an issue currently in progress in European Union. Digestate appears to be an efficient fertilizer, bringing comparative or better yield than treatments with mineral fertilizers (Lošák et al., 2010).

In the case of tomato, using twice the recommended amount of digestate coming from the AD treatment of poultry litter was significantly better for fruit number, average mass and total mass than treatment with chemical and certified organic fertilizers. In addition, the total mass per tomato plant was more than three times higher than the mass obtained by using other fertilizing treatments (chemical and certified organic fertilizers) (Liedl *et al.*, 2015).

Studies on digestate made from the AD treatment of recycling kitchen garbage effluents showed that it can be used as a fertilizer for vegetables and is comparable to chemical fertilizers on the growth of spinach and komatsuna (i.e. Japanese mustard spinach) (Furukawa & Hasegawe, 2006).

Concerning the quality of vegetables grown on digestate, qualitative parameters are similar or even better than those grown on mineral fertilizers (Lošák *et al.*, 2010).

Chemical composition

The separation of digestate phases creates two products, a liquid and a fibrous material, named here respectively liquid digestate (L-digestate) and solid digestate (S-digestate).

The composition of the solid digestate can highly vary (Möller and Müller, 2012). The relatively high mineral N content of solids indicates a high potential for N losses during handling and application because of ammonia volatilization (Möller and Müller, 2012; OFEV *et al.*, 2007).

The use of digestate coming from the AD treatment of animal manures could bring significant changes to tomato cultivation, with increase in organic matter, available N, P and K, total N and P, electrical conductivity, and contents of amino acids, proteins, soluble sugars, β-carotene, tannins, and vitamins (Yo *et al.*, 2009).

In addition, digestate coming from the AD treatment of kitchen garbage effluents does not cause contamination of coliform group, *Escherichia coli*, *Fecal streptococci* or *Vibrio parahaemolyticus*, both in the soil or in spinach and komatsuna leaves (Furukawa and Hasegawe, 2006). However, heavy metals are conserved during the AD and issues can appear (ADEME, 2011).

The microbiological contamination highly depends on the raw material composition. To decrease risks of contamination by pathogenic microorganisms, the digestate must be hygienized.

Field test report 1: Liquid fertilizers

ABSTRACT

This report analyses the potential for usage of liquid fertilizers produced from digestate and through solid-state fermentation of biowaste within DECISIVE project. A selection of the best mineral and organic commercial fertilizers was made. Digestate best formulation was studied. Determination of the best treatments and comparative results are presented here.

Table of contents Liquid fertilizers

1. State-of-the-art

1.1. FERTILIZATION MANAGEMENT

In hydroponics, fertilizers can be either mineral or organic and may take a solid (powder to be mixed with water) or a liquid form.
Fertilizers efficiency is managed in hydroponics following electrical conductivity (EC) and pH. On one hand, the EC measures the quantity of nutrients in ionic form that are available to plants in a solution (Wortman, 2015) and is usually set between 1 and 3 mS/cm according to the crop and its stage. In aquaponic system, the nutrient solution is made of aquaculture effluents, which are characterized by lower EC: between 0.3 and 1.1 mS/cm (Wortman, 2015). On the other hand, pH influences both nutrients availability to plants (due to chemical reactions) and microbial activity that provides nitrates for crops growing through ammonia and nitrites transformation. The optimal pH is a balance between these two mechanisms (Wortman, 2015). In hydroponics, the target pH is usually between 5.5 and 6.5 for mineral fertilizers. In aquaponics, the targeted pH is 7 to ensure nitrification without jeopardizing plant uptake potential (Tyson *et al.*, 2004). In the event of pH rising, acid cannot be added to organic fertilizer, as it would kill microorganisms that are beneficial to turn the organic nutrients into minerals.

1.2. DIGESTATE, PRODUCT OF ANAEROBIC DIGESTION

In hydroponics, digestate is an effective nutrient source for high-quality vegetable production concerning yield and quality improvement and stress resistance. However, deliberate component regulation still needs to be developed for better yield and quality of vegetable due to the large variability of components of digestate (Liu *et al.*, 2009).
Besides, for lettuces grown in hydroponics with digestate coming from the AD treatment of poultry waste, increasing the effluent concentration changes the taste of lettuces by intensifying bitter characteristics (Leidl *et al.*, 2004).

1.3. BIOSTIMULANTS

Several biostimulants were tested in the Liquid fertilizers field test.

1.3.1. Biostimulants definition

Biostimulants are substances and materials that stimulate the processes of plants nutrition, independently from the nutritive elements they contain, to the only purpose of increasing one or several characteristics of the plants (Commission Européenne, 2016). For example, they can stimulate aerobic microorganisms to degrade the organic matter (as it happens in compost), they can feed beneficial microorganisms, they can be used against abiotic stress. Biostimulants are recognized and defined at the European scale. However, there is no existing procedure for their homologation, in opposition to the conventional fertilizers, with the RCE 2003/2003 (Regulation (EC) NO 2003/2003 relating to fertilizers). Since 2009, the European Commission engaged a reflexion to incorporate biostimulants in European regulations on fertilizers (2014, BIO by Deloitte and RITTMO Agroenvironnement). Different groups work on the homogenisation of the regulation, such as the Fertilizers Working Group (2019, European Commission). For now, the homologation procedure is country specific.

1.3.2. Biostimulants overview

For this study, a global analysis of biostimulants of interest has been done. From this analysis and based on the number of scientific reports found on the different biostimulants, and the previous tests done by Refarmers, biostimulants have been selected: it was decided to focus on humic acid, compost tea (CT), molasses and fungi: *trichoderma* and *rhizophagus irregularis.*

1.3.3. Compost tea

Compost tea comes from an infusion of compost in water where organic matter, microorganisms and nutrients are transferred from the compost to the solution. Compost tea can be prepared with or without additional oxygenation. Therefore, beneficial organisms, such as bacteria, fungi, protozoa and nematodes present in compost are also present in compost tea (Ingham, 2005). Ingham (2005) listed seventeen potential benefits of compost tea, including that it could improve the life in the soil, protect plants from pathogens, help water retention of the soil, enhance the nutritional quality of plant and improve plant growth.
Indeed, Hargreaves *et al.* (2008) have shown that compost tea brings effective nutrient amendments. Also, compost tea caused significant increase of yield and positive impact on health and vegetative status of plants subjected to natural leaf blights (Pane *et al.*, 2015).

Furthermore, compost seems to have biocontrol properties (On *et al.*, 2014). However, for some plant diseases (e.g. apple scab), the level of control is considered inadequate for conventional agriculture, but an important improvement in organic agriculture (Scheuerell and Mahaffee, 2002).
Also, the compost tea nutrients and microorganisms are influenced by compost/water ratio, extraction time, and the storage duration affects the microbial population (Islam *et al.*, 2016). Compost tea quality highly depends on the quality of the compost used. To foster the growth of bacteria and/or fungi present in compost tea, several products can be used such as molasses or humic acid.

1.3.4. Molasses

Molasses are coproducts of residues of syrup from the fabrication or refinement of sugar from sugar beet or sugar cane. Molasses used to make alcohol produce a residual mash. This residue can be used as a fertilizer equivalent to mineral fertilizer (Brouwers and Farinet). Combined with compost tea, simple sugars as molasses, encourage bacteria growth (Ingham, 2005).

1.3.5. Humic acid

Humic substances are natural substances belonging to the soil organic matter and resulting from the decomposition of dead cell materials and from the metabolic activity of soil microbes using these substrates. Humic acids are very resistant to decomposition and highly condensed (Ingham, 2005). They might benefit plant growth by chelating unavailable nutrients and buffering pH, presenting a buffer action between pH 5.5 and 8.0 (Pertusatti and Prado, 2007). Current evidence suggests that humic substances are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (Canellas *et al.*, 2015). Lee and Bartlett (1976) reported that application of humic substances increases dry matter yield of corn seedlings.
There is a large range for the application of humic acids, including the combined use with compost tea, which enhances the growth of fungi (Ingham, 2005).

1.3.6. Trichoderma

Trichoderma are free-living fungi, commonly found in soil and root ecosystems. *Trichoderma* increases roots growth and can increase biofertilizer activity. Fungi increase nutrient uptake; the efficiency of nitrogen use and can solubilize nutrients in the soil. Nzanza *et al.* (2012) have shown that *Trichoderma harzianum* increases total yield of tomatoes grown in a greenhouse compared to treatments uninoculated. Also, in hydroponics Zhang et al. (2014) demonstrated that inoculation of *Trichoderma harzianum* increase the biomass of cucumber plants.
In addition, the fungus stimulates the microbial flora and protects plants from other fungi (Zhang *et al.*, 2014; Nzanza *et al.*, 2012; López-Bucio *et al.*, 2015; Benítez *et al.*, 2004). It is efficient as a biocontrol when installed before other fungi that are pathogens, meaning that it has a preventive action (Caron, 2002). In addition, it can also help plants to overcome abiotic stresses (Harman *et al.*, 2004; Zaidi *et al.*, 2017).

1.3.7. Rhizophagus irregularis

Rhizophagus irregularis is a well-known arbuscular mycorrhizal fungus (AMF). AMF supports plant nutrition with their capacity to improve plant mineral uptake (Smith and Read, 2008). AMF can boost plant secondary metabolism leading to improved nutraceutical compounds and can also confer tolerance to drought and adverse chemical soil conditions (Rouphael *et al.*, 2015). In addition, it has chelating properties (Rouphalel *et al.*, 2015; Porcel *et al.*, 2007; Avio *et al.*, 2017). As with *Trichoderma*, *Rhizophagus irregularis* increases the total yield of tomatoes grown in a greenhouse in comparison with treatments uninoculated and increases the percentage of extra-large fruit (Nzanza *et al.*, 2012).

2. Objectives

During Season 2018, three categories of liquid fertilizers were studied independently from each other: mineral fertilizers, organic fertilizers and bioproducts from AD and SSF (L-digestate and L-SSF). For each category three consecutive trials were designed focusing on adapted biostimulants and EC. The general objective was first to find the most suitable conditions for the bioproducts derived from the DECISIVE project. Then to find the best conditions for an organic and a mineral fertilizer to compare them to DECISIVE bioproducts

2.1. MINERAL FERTILIZERS

- Trial 1 analysed the best fertilizer and its most suitable EC within three mineral fertilizers.
- Trial 2 studied the most suitable EC combined or not with compost tea for two fertilizers.
- Trial 3 explored the most efficient EC for the best fertilizer.

2.2. ORGANIC FERTILIZERS

- Trial 1 studied the most suitable EC and fungal product within three organic fertilizers.
- Trial 2 explored the effect of compost tea and molasses on the best fertilizer and studied its shelf life.
- Trial 3 analysed a larger number of molasses concentration with or without compost tea on the best fertilizer.

Fungal products were studied because they were recommended by one of the providers or well known in agriculture. A more detailed investigation focused on compost tea and molasses as previous studies proved them to be efficient as biostimulants and interesting to combined. Indeed, compost tea brings microorganisms to the nutrient solution and molasses feed microorganisms.

2.3. BIOPRODUCTS FROM AD AND SSF

- Trial 1 investigated the most efficient EC and fungal product on L-digestate.
- Trial 2 explored the impact of compost tea and molasses on L-digestate and L-SSF.
- Trial 3 studied a larger range of molasses concentration with or without humic acid on L-digestate.

As for choice of biostimulants, same perspective as for “Organic fertilizers” was applied. Fungal product were tested first, then combined with compost tea and molasses. Humic acid was tested at the end as it is supposed to boost compost tea effect. Indeed, compost tea contains already some humic acids.

3. Materials and methods

3.1. CROPS

Blond Oak leaf lettuces (*Lactuca sativa)* were studied. Lettuces were chosen because of their quick growing cycles (four to seven weeks), allowing to operate several trials on the same installation during the given time and because nutrient deficiencies can be easily detected on lettuces (variations of mass, diameter and colour). Lettuces are also well-documented in hydroponics.

3.2. FERTILIZERS

In total, eight fertilizers were tested and classified in three categories: mineral fertilizers, organic fertilizers and bioproducts from AD and SSF (L-digestate and L-SSF). Origin and preparation of each of them are described in Table 1.

.

Table 1: List of the fertilizers studied in Liquid fertilizers field test

Fertilizer	Category	Origin	Preparation
Pm A*	Mineral fertilizer	commercial	Dilution. Ratio ½ fertilizer and ½ calcium nitrate
Pm B	Mineral fertilizer	commercial	Dilution. Ratio ½ fertilizer and ½ calcium nitrate
Pm C	Mineral fertilizer	commercial	Dilution with water to reach the target EC
Po A**	Organic fertilizer	commercial	Dilution with water to reach the target EC
Po B	Organic fertilizer	commercial	Dilution with water to reach the target EC
Po C	Organic fertilizer	In-trial	Filtration of the solid part and dilution with water to reach the target EC
L-digestate	Bioproduct from biowaste through AD	Partners IRSTEA and Suez	Dilution with water to reach the target EC
L-SSF	Bioproduct from biowaste through SSF	Partner UAB	Dilution with water to reach the target EC

*Pm for Product mineral
**Po for Product organic

3.3. BIOSTIMULANTS

In total, five biostimulants were tested and classified in four categories: fungal products, compost tea, molasses and humic acid.

Table 2: List of the biostimulants studied in Liquid fertilizers field test

Biostimulant	Category	Origin	Preparation
F1	Fungal product	Commercial powder based on *Trichoderma harzianum*	Dilution in the nutrient solution
F2	Fungal product	Commercial powder based on *Rhizophagus irregularis* and *Trichoderma atroviride*	Dilution in the nutrient solution
Compost tea	Compost tea	Commercial solution	Oxygenation then dilution in the nutrient solution
Molasses	Molasses	Commercial solution	Dilution in the compost tea (if present in the treatment) or directly in the nutrient solution
Humic acid	Humic acid	Commercial solution	Dilution in the compost tea (if present in the treatment) or directly in the nutrient solution

3.4. HYDROPONICS SYSTEM SET-UP

Lettuces were planted in vertical hydroponics ZipGrow^{TM} (ZG) towers as shown on Figure 2. ZG towers are composed of a matrix media where roots develop and a wicking strip to orientate the water flow. It is made of recycled PET and is inert. Thus, it does not add nutrients to the solution. The

matrix media allows roots to grow and is an efficient filtration system, mechanical (by stopping untransformed organic particles) and biological (by hosting nitrifying bacteria). They are interesting because they work in aerobic system, allowing the development of microorganisms.
Towers were organized in lines of five with six lettuces evenly spread on each, which gives 30 lettuces per line. Each line corresponded to a statistic treatment: it had a barrel containing a nutrient solution independent from other lines as shown on
Figure 2 and Figure 3.

Figure 2: Experimental set-up of the Liquid fertilizers field test – photo

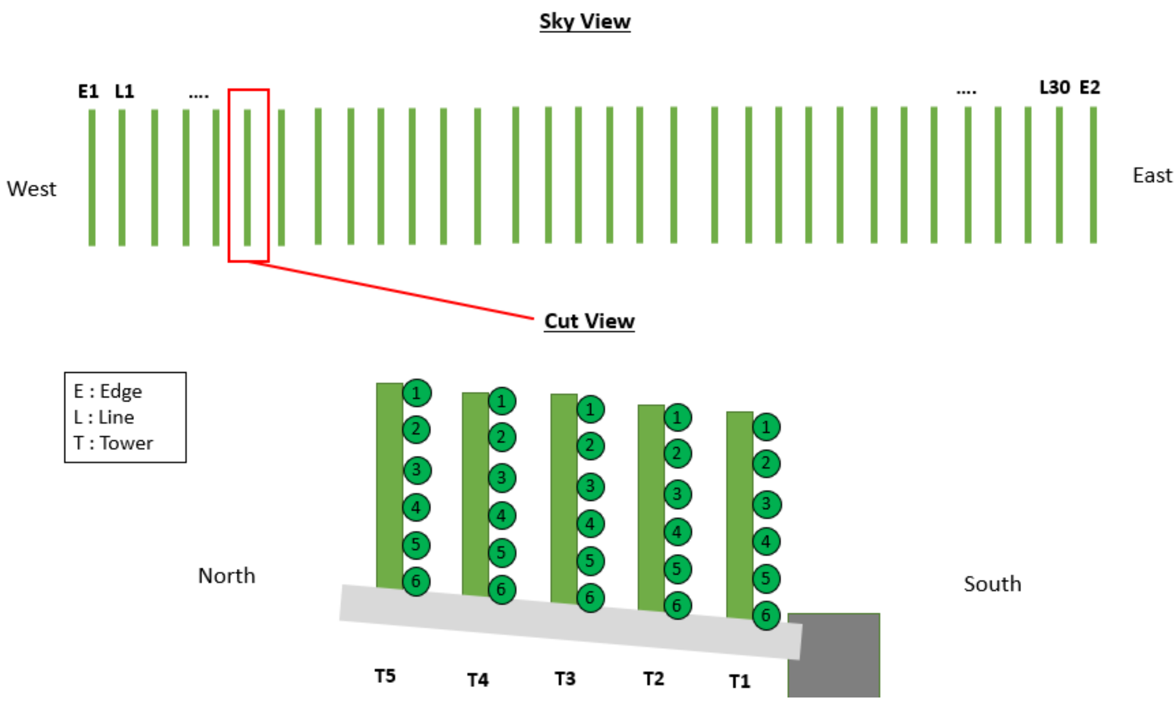


Figure 3: Experimental set-up of the Liquid fertilizers field test – scheme

3.5. FIELD TESTS

3.5.1. General features

Each barrel was run according to its independent protocol. Water was filled up and fertilization was added at the beginning of the growing cycle and if needed during the growing cycle.

Three trials were performed between April and October 2018 (Trials 1, 2, 3), each one corresponding to a growing cycle. A statistical analysis was then performed.
To study edge effect due to wind or specific weather conditions, two lines, called edge line (E) were added at the extremities of the experimentation as shown on Figure 3. To assess edge effect, lines E received the same fertilization as their neighbour lines.
EC and pH measured in the routine were recorded with portable EC meters and portable pH meters. Calibration was done before each measure for pH meters and every month for EC meters.
Fresh matter mass of lettuces and their diameters values were recorded at harvest as quantitative data to assess efficiency of the treatments. The diameter was measured directly on the tower, just before harvest, using the average between the width and the length of lettuces. Each treatment had 30 replicates corresponding to the 30 lettuces per line.
Materials and system were cleaned and disinfected with black soap and 2 % diluted bleach solutions between each trial after harvesting and data collection. Then, they were rinsed with clear water to avoid the presence of bleach leftover which could have affected the growth of lettuces for the next trial.
Data recorded were compared within the same trial (growing cycle), but not between trials as it would give too much variation because of different climate conditions.

3.5.2. Mineral fertilizers

For mineral fertilizers, the experimental design was performed as detailed in Table 3

Table 3: List of the mineral fertilizer treatments in Liquid fertilizers field test

Trial	Treatment n°	Fertilizer	EC (mS/cm)	Biostimulant
Trial 1- April 18	1	Pm A	0.8	-
	2	Pm A	1.2	-
	3	Pm B	0.8	-
	4	Pm B	1.2	-
	5	Pm C	0.8	-
	6	Pm C	1.2	-
Trial 2 - June 2018	31	Pm B	1	-
	32	Pm B	1	CT*
	33	Pm B	1.2	-
	34	Pm B	1.2	CT
	35	Pm B	1.4	-
	36	Pm B	1.4	CT
	37	Pm C	1	-
	38	Pm C	1	CT
	39	Pm C	1.2	-
	40	Pm C	1.2	CT
	41	Pm C	1.4	-
	42	Pm C	1.4	CT
Trial 3 – August 2018	61	Pm C	1.3	-
	62	Pm C	1.4	-
	63	Pm C	1.5	-

	64	Pm C	1.6	-
	65	Pm C	1.7	-
	66	Pm C	1.8	-

*CT for Compost Tea

For each trial, target pH was between 5.5 and 6.5 for mineral solutions. If pH raised, acid was added to reach the target pH.

3.5.3. Organic fertilizers

For organic fertilizers, the experimental design was performed as detailed in Table 4:

Table 4: List of the organic fertilizer treatments in Liquid fertilizers field test

Trial	Treatment n°	Fertilizer	EC (mS/cm)	Lifespan	Biostimulant
Trial 1- April 18	7	Po A	0.8	-	-
	8	Po A	0.8	-	F1
	9	Po A	0.8	-	F2
	10	Po A	1	-	-
	11	Po A	1	-	F1
	12	Po A	1	-	F2
	13	Po B	1	-	-
	14	Po B	1	-	F1
	15	Po B	1	-	F2
	16	Po B	1.2	-	-
	17	Po B	1.2	-	F1
	18	Po B	1.2	-	F2
	19	Po C	1	-	-
	20	Po C	1	-	F1
	21	Po C	1	-	F2
	22	Po C	1.2	-	-
	23	Po C	1.2	-	F1
	24	Po C	1.2	-	F2
Trial 2 - June 2018	43	Po B	1.2	-	F1, M* 0 ml/l
	44	Po B	1.2	-	F1, M 0.5 ml/L
	45	Po B	1.2	-	F1, M 0.25 ml/L
	46	Po B	1.2	-	F1, CT**, M 0 ml/l
	47	Po B	1.2	-	F1, CT, M 0.5 ml/l
	48	Po B	1.2	-	F1, CT, M 0.25 ml/l

Trial 3 – August 2018	67	Po B	1.2	-	F1, M 0 ml/l
	68	Po B	1.2	-	F1, M 0.15 ml/l
	69	Po B	1.2	-	F1, M 0.25 ml/l
	70	Po B	1.2	-	F1, M 0.35 ml/l
	71	Po B	1.2	-	F1, M 0.5 ml/l
	72	Po B	1.2	-	F1, CT, M 0 ml/l
	73	Po B	1.2	-	F1, CT, M 0.15 ml/l
	74	Po B	1.2	-	F1, CT, M 0.25 ml/l
	75	Po B	1.2	-	F1, CT, M 0.35 ml/l
	76	Po B	1.2	-	F1, CT, M 0.5 ml/l
	77	Po B	1.2	1 day old	F1
	78	Po B	1.2	2 months old	F1
	79	Po B	1.2	4 months old	F1

*M for Molasses
**CT for Compost Tea

If pH raised in organic solutions, acid was not added to avoid killing useful microorganisms and thus pH was not controlled. EC values, compost tea quantity and molasses concentrations tested were chosen according to the literature or to suppliers' recommendations.

3.5.4. Bioproducts from AD and SSF

The experimental design was performed as detailed in Table 5.

Table 5: List of the bioproducts from AD and SSF treatments in Liquid fertilizers field test

Trial	Treatment n°	Fertilizer	EC (mS/cm)	Biostimulant
Trial 1- April 18	25	L-digestate	1	-
	26	L-digestate	1	F1
	27	L-digestate	1	F2
	28	L-digestate	1.2	-
	29	L-digestate	1.2	F1
	30	L-digestate	1.2	F2
Trial 2 - June 2018	49	L-digestate	1.2	F2, M* 0 ml/l
	50	L-digestate	1.2	F2, M 0.5 ml/l
	51	L-digestate	1.2	F2, M 0.25 ml/l
	52	L-digestate	1.2	F2, CT**, M 0 ml/l
	53	L-digestate	1.2	F2, CT, M 0.5 ml/l
	54	L-digestate	1.2	F2, CT, M 0.25 ml/l
	55	L-SSF	1.2	F2, M 0 ml/l

	56	L-SSF	1.2	F2, M 0.5 ml/l
	57	L-SSF	1.2	F2, M 0.25 ml/l
	58	L-SSF	1.2	F2, CT, M 0 ml/l
	59	L-SSF	1.2	F2, CT, M 0.5 ml/l
	60	L-SSF	1.2	F2, CT, M 0.25 ml/l
Trial 3 – August 2018	80	L-digestate	1.2	F2, CT, M 0 ml/l
	81	L-digestate	1.2	F2, CT, M 0.25 ml/l
	82	L-digestate	1.2	F2, CT, M 0.35 ml/l
	83	L-digestate	1.2	F2, CT, M 0.5 ml/l
	84	L-digestate	1.2	F2, CT, M 0.6 ml/l
	85	L-digestate	1.2	F2, CT, M 0.7 ml/l
	86	L-digestate	1.2	F2, CT, M 0.25 ml/l, HA***
	87	L-digestate	1.2	F2, CT, M 0.35 ml/l, HA
	88	L-digestate	1.2	F2, CT, M 0.5 ml/l, HA
	89	L-digestate	1.2	F2, CT, M 0.6 ml/l, HA
	90	L-digestate	1.2	F2, CT, M 0.7 ml/l, HA

*M for Molasses
**CT for Compost Tea
***HA for Humic Acid

In bioproducts solutions, acid was not added to avoid killing useful microorganisms and thus pH was not controlled and ranged from 7.5 to 8.5. EC values, compost tea quantity, molasses concentrations and humic acid quantity tested were chosen according to the literature or to suppliers' recommendations.

3.6. STATISTICAL METHODS

The objective was to compare the mass and diameter of lettuces to show if there were or not significant differences between the studied treatments. Firstly, data were cleaned: values were taken out of data in case of clogged irrigation that resulted in dead or weak lettuces, or in case of disease. Then, multiple test comparisons, simple test comparisons, or linear regressions were performed.

3.6.1. Multiple test comparison

ANOVA were conducted to determine if there were significant influence of treatments on mass and diameter of lettuces knowing that samples were independent (with 30 replicates). Shapiro-Wilk test and Levene's tests were conducted to verify the condition of application of the ANOVA (normality of residuals and homogeneity of variance).

The hypothesis Ho was always: “there was no significant difference between the studied treatments”. The alternative hypothesis H1 was “at least one of the treatments led to values of the studied variable significantly different from the others”. The acceptance limit of Ho was $\alpha = 5$ %. In case the results of ANOVA gave p-value <0.05, the Tukey's HSD test was conducted to find which treatment(s) resulted in values significantly different from the others.

In case of normality but non-homogeneity of variances, a Welch ANOVA was conducted, followed by a post-hoc Games-Howell test. In case of non-normality, a non-parametric test of Kruskal-Wallis was run, followed by a Wilcoxon-Mann-Whitney test with the Bonferroni adjustment for multiple comparisons.

In the report, results are visualized using boxplot on which means of each treatment are illustrated in blue, 95 % confidence intervals in red and mean of all treatments in green.

Limit of box corresponds to the lower and upper quartiles. Whiskers were defined using the following formulas:

$$\text{Upper whisker} = \min(\max(x), Q3 + 1.5 * IQR)$$

Lower whisker = max (min(x), Q1 – 1.5 * IQR)
where Q1 is the lower quartile, Q3 the upper quartile and IQR = Q3-Q1

3.6.2. Simple test comparison

Simple test comparison was applied when only two comparisons were performed: to measure the edge lines to their neighbour lines. Shapiro-Wilk test was run to assess normality and Fisher test to assess the homogeneity of variance. As the samples were independent and there were 30 replicates, a Z-test was conducted. The hypothesis Ho and H1 were identical as for the multiple test comparison and the significance threshold was $\alpha = 5$ %.
In case of non-homogeneity of variance, a Student t-test was conducted. In case of non-normality, a Wilcoxon-Mann-Whitney test was run.

3.6.3. Linear regression

Linear regression was performed when only one modality was studied in the treatments: different EC on a mineral fertilizer. It evaluated the significance of the linear relationship between the two variables: EC and mass or EC and diameters. Before the regression, a visual evaluation of the linearity was done. After the regression, hypothesis of validity were verified: normality and independence of the residuals and homogeneity of variance. In the report, the regression is represented with the slope and associated confidence interval at 95 %. In addition, the prediction interval at 95 % was added to the graphic.

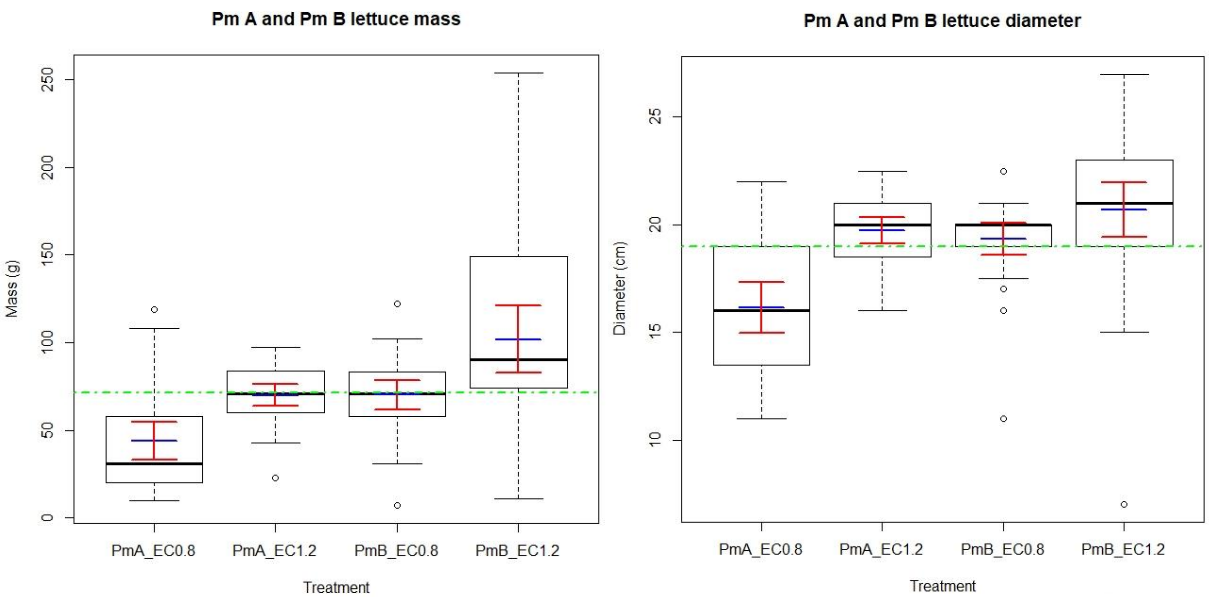
4. Results and discussion

4.1. MINERAL FERTILIZERS

The fertilizing potential of three mineral fertilizers Pm A, Pm B and Pm C was compared, at different EC and with compost tea (CT) added or not. P-values are not indicated, for additional information about the p-values, please see the Annex 1, where they are all referred.

4.1.1. Trial 1: fertilizers and EC

During Trial 1 of mineral fertilizers, an accident occurred on Pm: an excess of acid was added once during the nutrient solution management, which may have caused stress on the plant and modified mass and diameters. Thus, Pm C was studied later in Trial 2 and only Pm A and Pm B are discussed in Trial 1. Results are presented in Figure 4.



- PmB-EC1.2 gave mass significantly higher than PmB-EC0.8 and PmA-EC1.2.
- PmB-EC1.2 gave diameter significantly higher than PmB-EC0.8, PmA-EC1.2 and PmA-EC0.8.

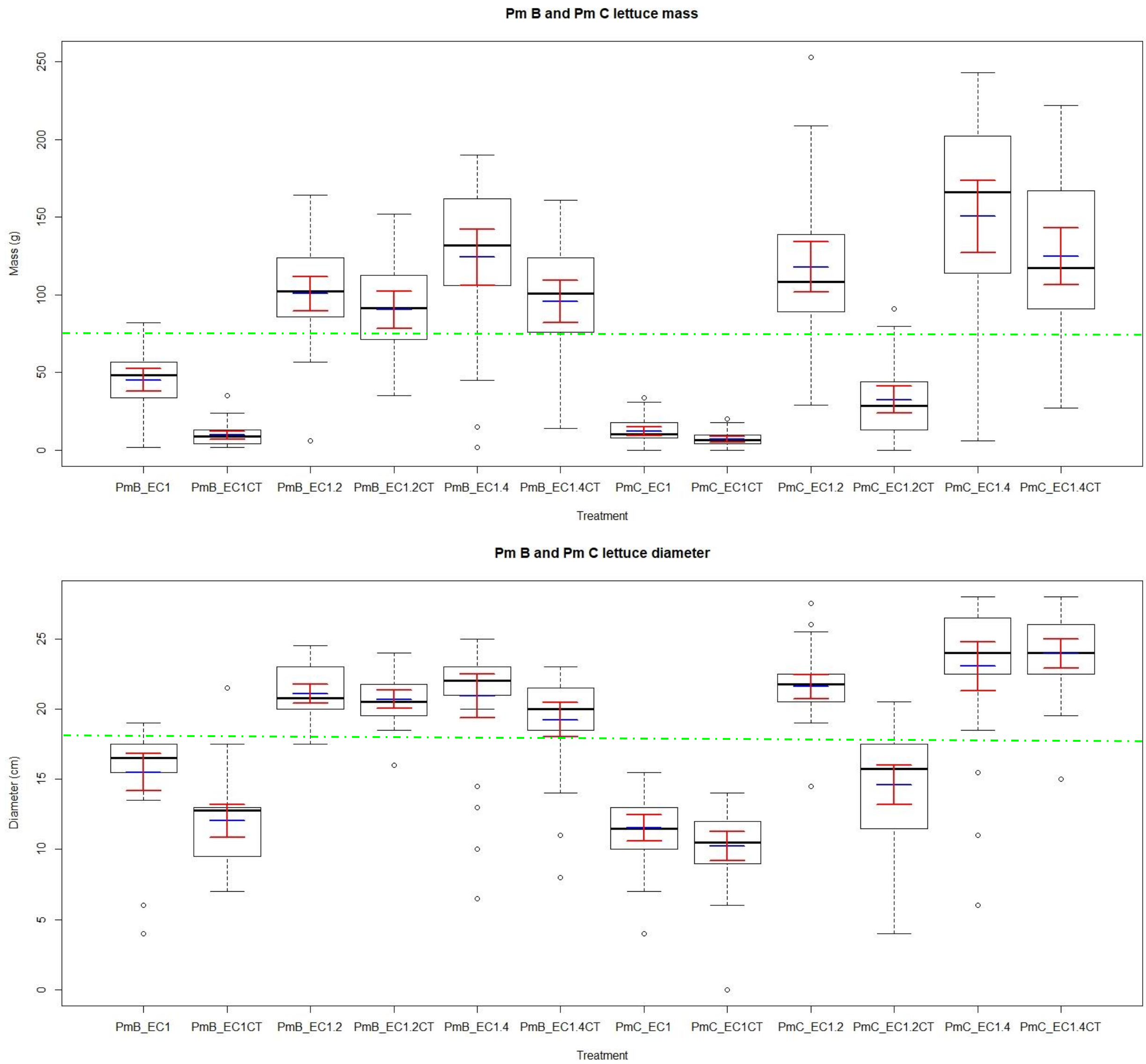
Figure 4: Trial 1- Mineral fertilizers - lettuces mass and diameter

In Trial 1, the fertilizer Pm B at the EC 1.2 mS/cm resulted in lettuces with mass (mean 102 g) and diameter (mean 21 cm) higher than found with the fertilizer Pm A (mean 57 g and 18 cm).

Thus, it was decided to keep in the next trial Pm B as the most efficient and Pm C due to the accident.

4.1.2. Trial 2: Compost tea and EC

Results are presented in Figure 5.



- For Pm B: PmB-EC1.2, PmB-EC1.2 CT, PmB-EC1.4 and PmB-EC1.4 CT gave mass and diameters significantly higher than PmB-EC1 and PmB-EC1 CT.
- For Pm C: PmC-EC1.2, PmC-EC1.4 and PmC-EC1.4 CT gave mass and diameter significantly higher than PmC-EC1.2 CT.
- For both: PmC-EC1.4 gave mass and diameter significantly bigger than all the treatments of Pm B except PmB-EC1.4.

Figure 5: Trial 2 - Mineral fertilizers - lettuces mass and diameter

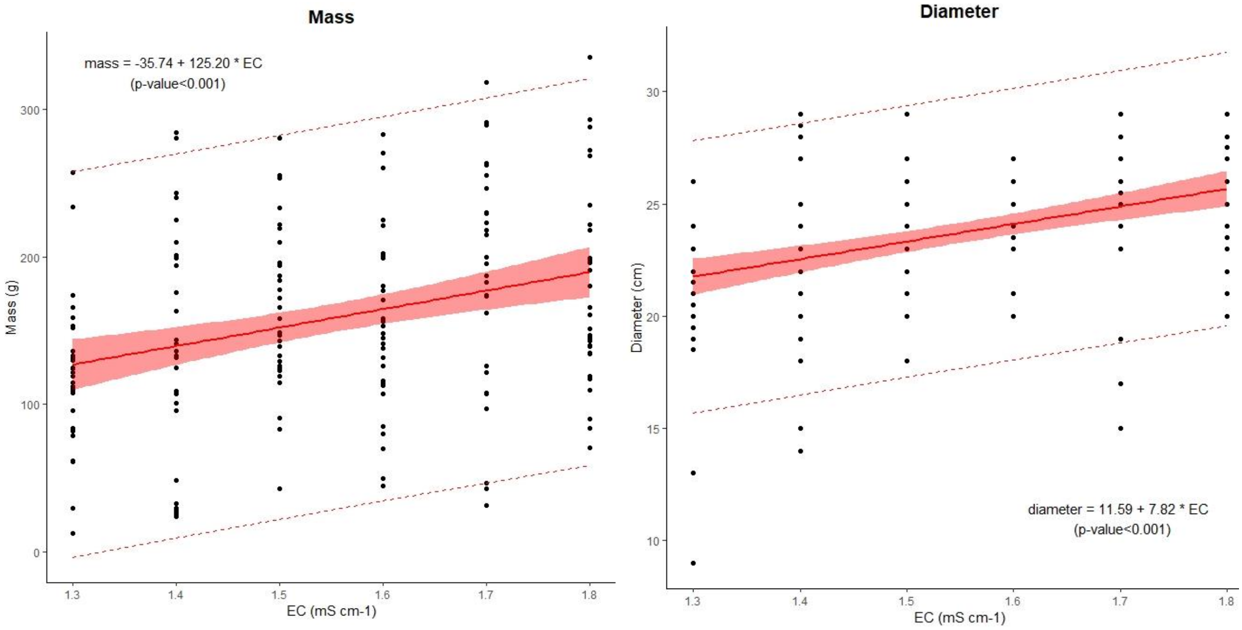
In Trial 2, higher EC resulted in heavier (mass) and larger (diameter) lettuce. The addition of compost tea had disadvantageous consequences on the lettuces that, on average, were significantly lighter and smaller than without compost tea. The fertilizers Pm B and Pm C at the maximum EC of 1.4 mS/cm and without compost tea, resulted in lettuces with mass (means 124 and 150 g) and diameters (means 21 and 24 cm) higher than the other treatments. There was no significant difference between Pm B and Pm C at the highest EC, 1.4 mS/cm.

EC values were higher in Trial 2 than in Trial 1 and, as before, the highest EC values gave the best results. Thus, EC seemed the most relevant parameter to regulate and EC effect was studied more in detail in Trial 3. Pm C was the only mineral fertilizer kept in Trial 3 as number of treatments per growing cycle were limited.

4.1.3. Trial 3: EC

- **Statistical results**

Results are presented in Figure 6.



Dotted lines: prediction interval at 95%
Shaded areas: confidence interval at 95% level

Figure 6: Trial 3 - Pm C – linear regression on lettuces mass and diameter with EC.

In Trial 3, Pm C with the EC 1.7 mS/cm gave the biggest mass (mean 193 g) and diameters (mean 26 cm). Regarding statistical results, mass and diameter were linearly corelated to the EC. When EC increases of 0.1 mS/cm the mass increases of 12.5 g and diameter of 0.78 cm. EC 1.7 was not significantly different from EC 1.8 mS/cm.

4.1.4. Discussion

- **Best fertilizers**

Statistical results concluded that Pm C and Pm B gave the highest diameters and mass for low EC values. Thus, they were considered as better fertilizers than Pm A. However, Pm B was not studied in Trial 3 due to limited equipment and space. Then, further analyses will be needed to compare whether Pm C or Pm B gives better growing performances at higher EC.

- **EC impact**

Higher EC gave better results in Trial 1 (1.2 mS/cm) and 2 (1.4 mS/cm) for Pm C and Pm B. In Trial 3, 1.7 mS/cm was the best EC for Pm C. However, increasing the EC have certainly impacted the nitrate content in the lettuces. In order to validate if the lettuces followed European regulation in term of nitrate level, laboratory analysis would have been needed.

- **Compost tea impact**

Even if the results were not statistically significant for all treatments, compost tea had a negative impact on average mass and diameters for the same EC. Thus, mineral fertilizers were more efficient without compost tea. It can be supposed that adding compost tea to the nutritive solution also increased the EC whereas fertilizer concentration did not change. Mineral fertilizers were managed using the EC. Thus, we might have put a lower quantity of fertilizer in the treatments combined with compost tea.

4.2. ORGANIC FERTILIZERS

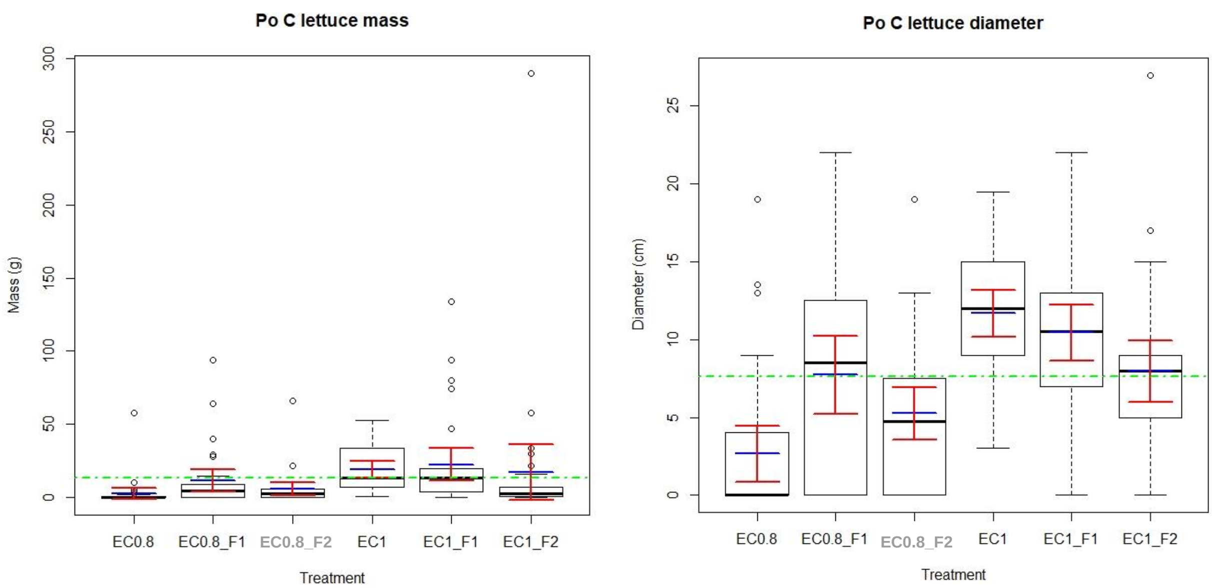
The fertilizing potential of three organic fertilizers: Po A, Po B and Po C, was compared with different EC and with different biostimulants added. P-values are not indicated, for additional information about the p-values, please see the Annex 1, where they are all referred.

4.2.1. Trial 1: fertilizers, EC and fungi

- **Po C**

Po C results are presented in Figure 7 and Figure 8.

Figure 7: Trial 1 - Po C – photo of lettuces



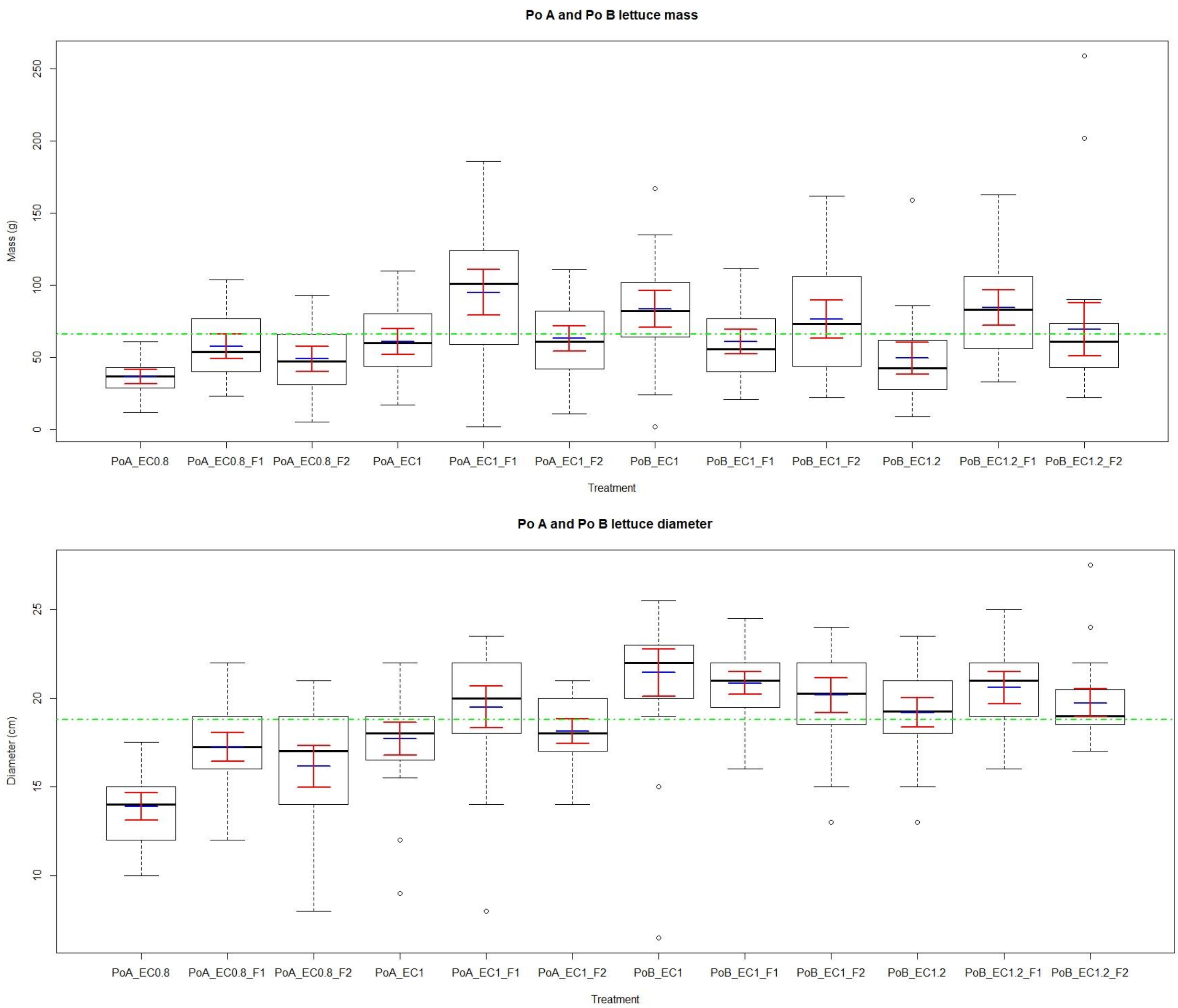
- EC1 gave mass significantly higher than EC0.8, EC0.8 F1, EC0.8 F2 and EC1 F2.
- EC1 F1 gave mass significantly higher than EC0.8 F2.
- EC1 gave diameter significantly higher than EC0.8, EC1 F2 and EC1 F2.
- EC1 F1 gave diameter significantly higher than EC0.8 F2 and EC0.8.

Figure 8: Trial 1- Po C - lettuces mass and diameter

In Trial 1 on Po C fertilizer, EC 1 mS/cm gave mass (mean 19 g) significantly bigger than other treatments. However, all treatments resulted in deficient lettuces lacking nutrients and lots of heterogeneity with both small and large lettuces. More than half of the lettuces were very small or dead. Therefore, it was decided to stop studying Po C. However, it is intriguing to notice that the biggest lettuce of all the organic fertilizers of Trial 1 was within the treatment EC1 F2 with a mass of 290g.

- **Po A and Po B**

Po A and Po B Trial 1 results are shown in Figure 9.



- <u>For Po A</u>: PoA-EC1 F1 gave mass significantly higher than all the Po A.
- <u>For Po A</u>: PoA-EC1 F1 gave diameter significantly higher than all the treatment at EC0.8.
- <u>For Po B</u>: PoB-EC1 gave mass and diameter significantly higher than PoB-EC1.2.
- <u>For Po B:</u> PoB-EC1.2 F1 gave mass significantly higher than PoB-EC1.2.
- <u>For both</u>: PoA-EC1 F1 gave mass significantly higher than PoB-EC1.2.
- <u>For both</u>: PoA-EC1 F1 gave diameter not significantly different than all the Po B treatments

Figure 9: Trial 1 - Po A & Po B - lettuces mass and diameter

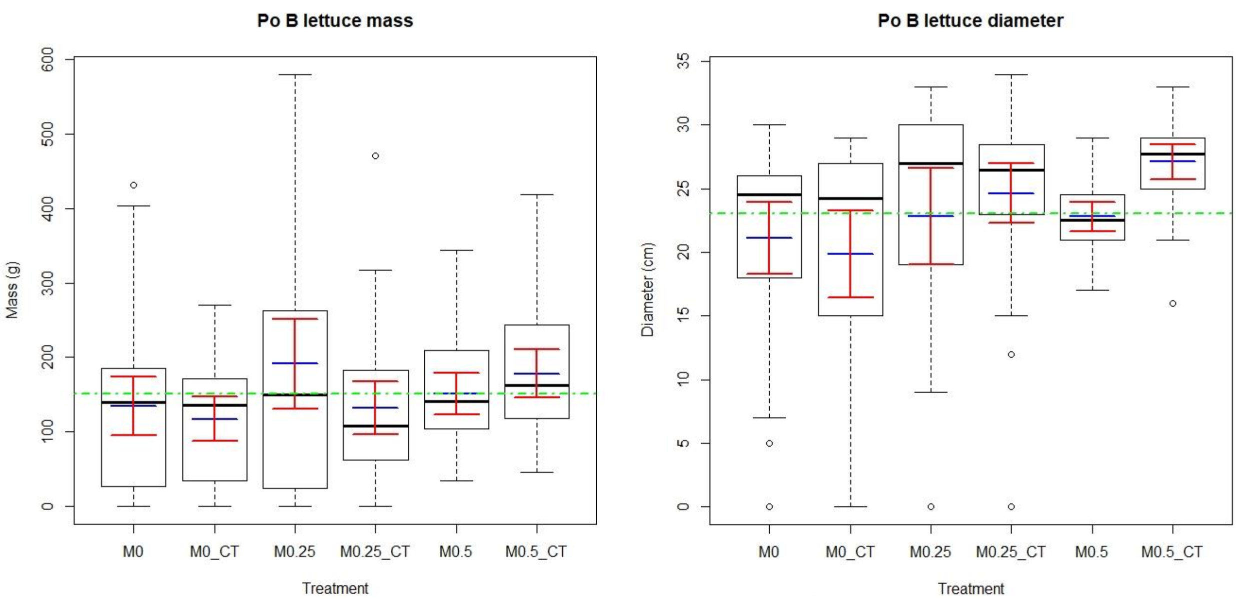
Mass and diameter results were quite heterogeneous for the different lettuces. However, results were not significantly different for the treatments applied.

Only one fertilizer was kept in Trial 2 as the number of treatments that could be carried out in Trial 2 was limited. Po B (with F1) was chosen over Po A as the lettuce texture and aesthetic were closer to mineral hydroponics. Indeed, Po A lettuces were very compact and with very thick leaves, which consumers are not used to.

4.2.2. Trial 2: Po B - Compost tea and molasses

Results are presented in Figure 10 and Figure 11.

Figure 10: Trial 2 - Po B – photo of lettuces



- There was no treatment that gave mass significantly different from the others.
- M0.5_CT gave diameters significantly higher than M0, M0 CT and M0.5.

Figure 11: Trial 2 – Po B - lettuces mass and diameter

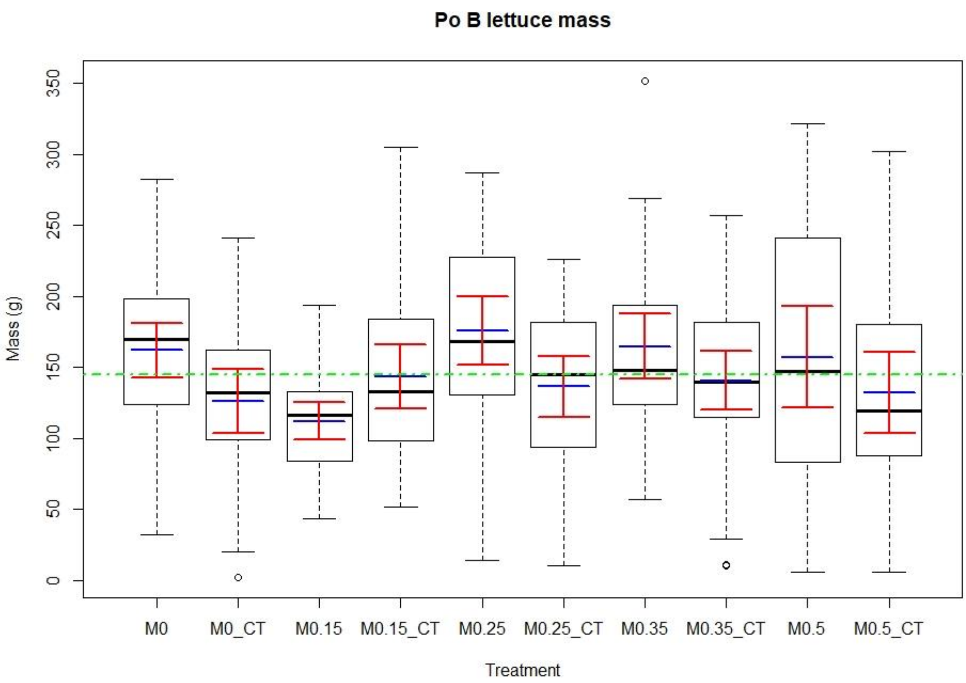
There were no significant differences between treatments regarding lettuces mass, but treatment with compost tea and a molasses concentration of 0.5 ml/l resulted in higher diameter (mean 27 cm) than the other treatments.

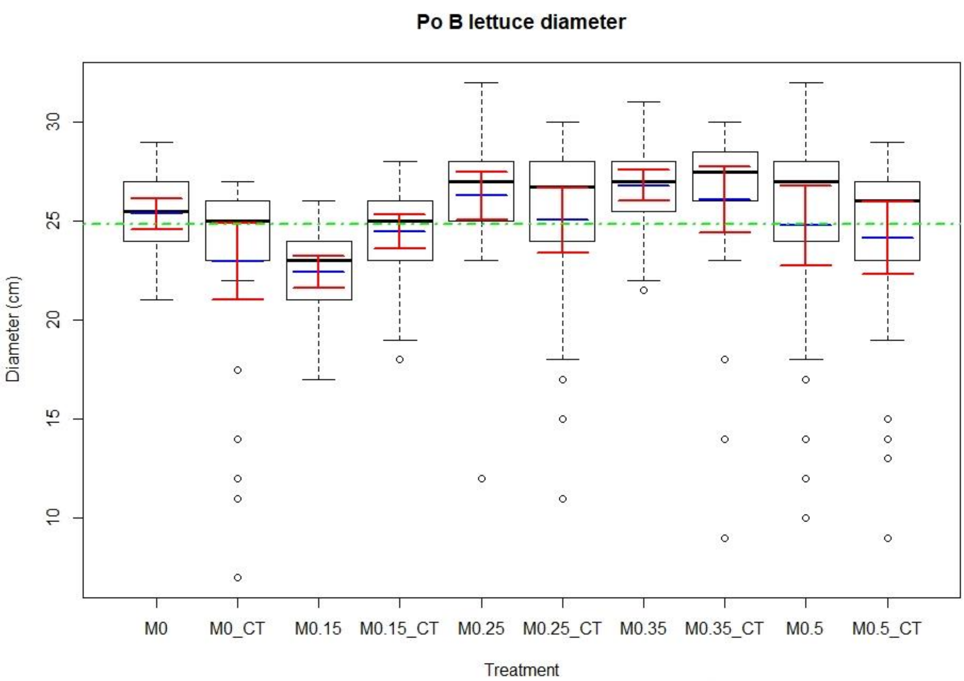
According to these results, it seemed that the molasses concentration influenced the lettuces diameter. Thus, it was decided to study the impact of various concentrations of molasses in the following trial.

4.2.3. Trial 3: Po B – Biostimulants and lifespan

- **Biostimulant**

Results are presented in Figure 12.





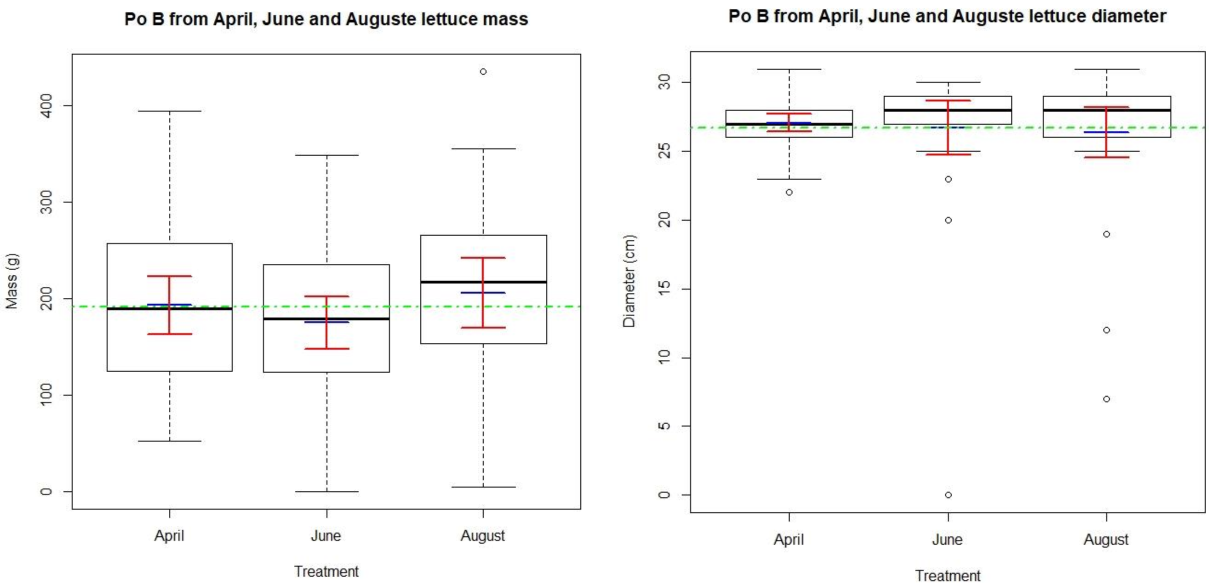
- M0.15 gave mass significantly smaller than M0, M0.25 and M0.35.
- M0.15 gave diameters significantly smaller than all other treatment except M0_CT.
- M0.15_CT and M0_CT gave diameters significantly smaller than M0.25, M0.35 and M0.35_CT.

Figure 12: Trial 3 - Po B - lettuces mass and diameter

In Trial 3, presence of compost tea and molasses did not significantly increse masses of lettuces. However, the treatment with a concentration of molasses at 0.15 ml/l and no compost tea gave diameter (mean 26 cm) of lettuces smaller than several other treatments.

- **Lifespan of Po B**

Results are presented in Figure 13.



- For both mass and diameter, there was no significant difference between treatments

Figure 13: Trial 3 - Po B from April, June and August - lettuces mass and diameter

From the comparison of the lifespan of Po B was concluded that the lifespan of the three solutions opened respectively in April, June and August did not change the mass and diameter of the lettuces.

4.2.4. Discussion

- **Best fertilizers**

Among the studied organic fertilizers, Po B and Po A were the most efficient compared to Po C. This one was removed from the study because of the high heterogeneity of mass and diameters within the obtained lettuces. It was decided to keep studying Po B more than Po A. Indeed, Po B lettuce texture and aesthetic were closer to mineral hydroponics. Po A lettuces were very compact and with very thick leaves, which consumers are not used to.

- **EC**

Po B was used at higher EC than Po A. Indeed, Po A EC was managed following the manufacturer recommendation of keeping it between 0.8 and 1 mS/cm but Po B EC have been increased as nutrient deficiencies were observed at the beginning of the trial at the recommended EC.
Po B difference of EC impact was variable according to the fungi used whereas higher EC gave better results for Po A at EC 1 mS/cm. A hypothesis is that increasing Po A EC could have improved its efficiency.

- **Fungi**

For Po A fertilizer, adding F1 to the nutritive solution had a positive impact on the mass and diameter of lettuces. However, for the Po B fertilizer the effects were not significantly different than without F1.
Indeed, F1 was the only biostimulant recommended by the manufacturer for Po A.
Concerning F2, results were not concordant from a treatment to another at different EC. A hypothesis is that the commercial organic fertilizers already contain all the biostimulants necessary to the development of the plant, thus the fungal products did not influence the growth of plants. Po B contains 25 % of sugar cane molasse and other natural plant extracts. Concerning Po A, the content is not given by the provider.

- **Compost tea and molasses**

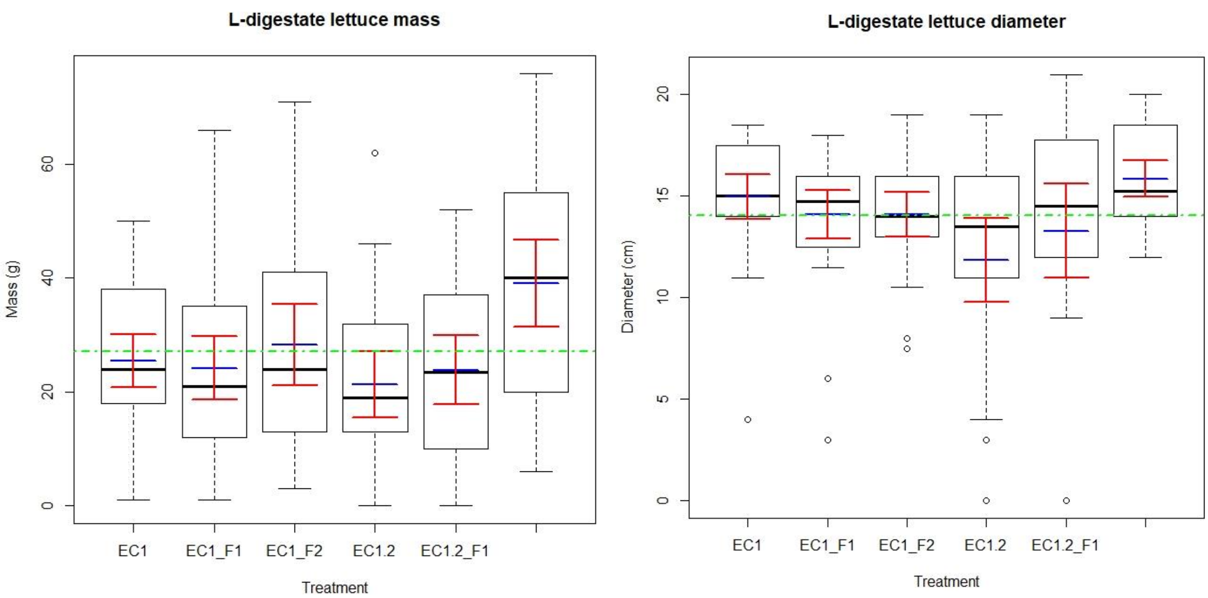
On organic fertilizer it was concluded that the presence of compost tea and molasses did not have effect on the mass of lettuces. However, some concentration of molasses with or without compost tea had a positive impact on the diameter of lettuces.
Molasses contain sugars and micronutrients (Ingham, 2005). A first hypothesis is that the sugar helped the development of microorganisms contained into the fertilizers. The second hypothesis is that the micronutrients in the molasses are different from those in the fertilizers, and that they complemented the fertilization. Concerning the compost tea that did not bring impact on the lettuces mass and diameters, it can be supposed that the commercial organic fertilizers contain already all the biostimulants the plants need to develop. Thus, compost tea did not bring any complement to the fertilization.

4.3. L-DIGESTATE AND L-SSF

The fertilizing potential of L-digestate and L-SSF was studied at different EC and considering different biostimulants. P-values are not indicated, for additional information about the p-values, please see the Annex 1, where they are all referred.

4.3.1. Trial 1: L-digestate EC and fungi

Results are presented in Figure 14.



- EC1.2 F2 gave mass significantly higher than EC1, EC1 F1, EC1.2 and EC1.2 F1.
- There was no significant difference between diameters for all treatments.

Figure 14: Trial 1 – L-digestate - lettuces mass and diameter

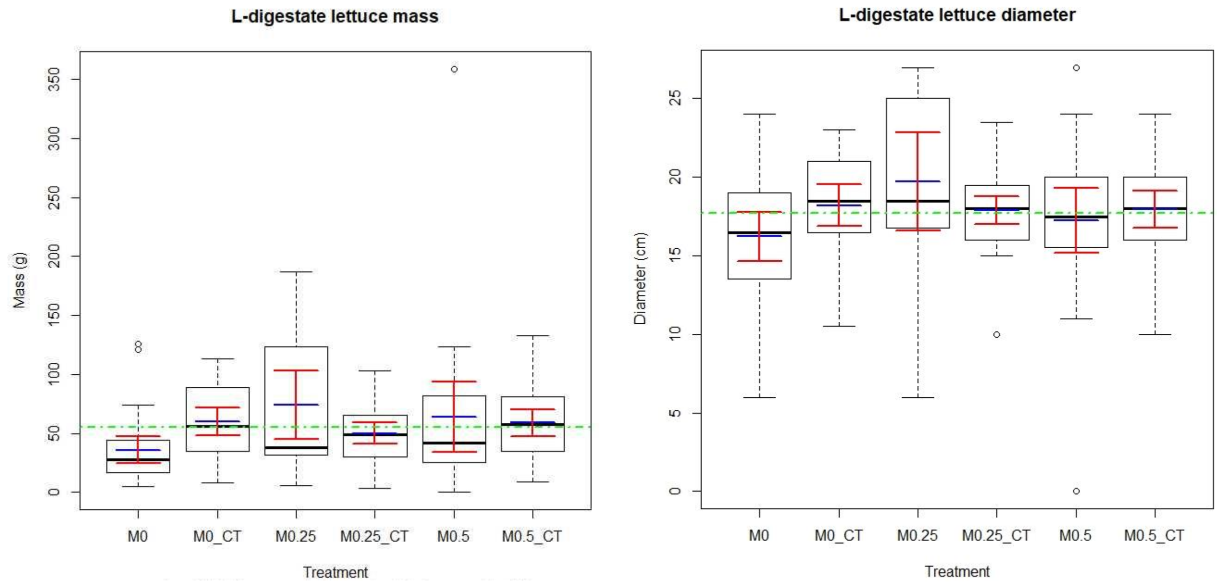
In Trial 1, the treatment EC1.2 with F2 gave mass of lettuces (mean 39 g) significantly bigger than all other treatments except EC1 with F2. There was no effect of treatments on the diameters of lettuces.

These results showed a relevant effect of F2 addition on L-digestate and it was decided to always add it to L-digestate in the following trial. The role of compost tea and molasses for L-digestate and L-SSF was then studied.

4.3.2. Trial 2: L-digestate and L-SSF compost tea and molasses

- **L-digestate**

Results are presented in Figure 15.



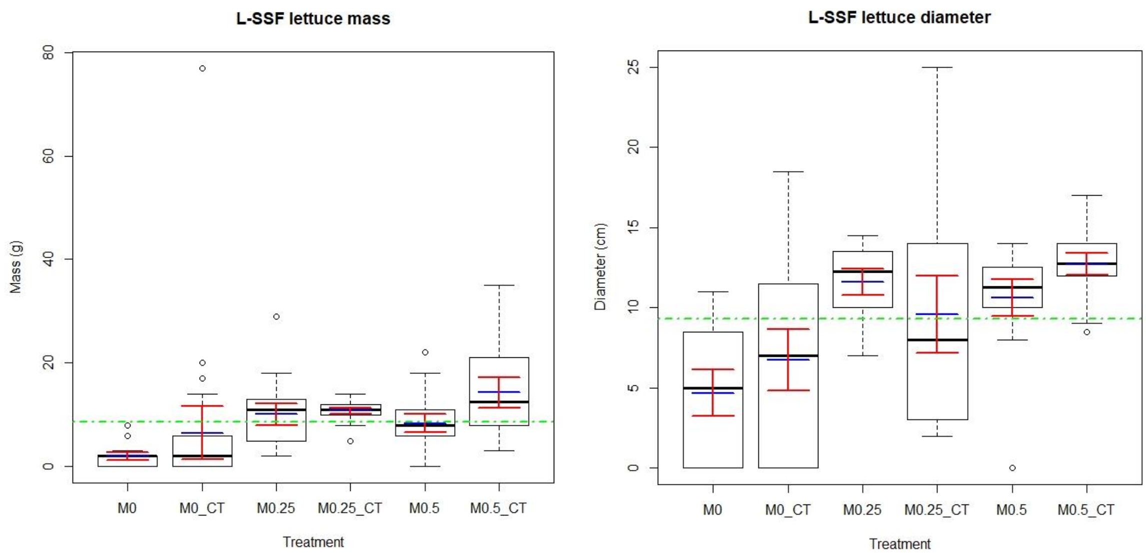
- M0.5_CT gave mass significantly higher than M0.
- There was no significant difference between diameters for all treatments.

Figure 15: Trial 2 – L-digestate - lettuces mass and diameter

In Trial 2, the treatment with compost tea and molasses at a concentration of 0.5 ml/l gave mass (mean 59 g) significantly higher than the treatment that does not contain compost tea and molasses (mean 36 g). Molasses and compost tea did not have a significant effect on the fertilizing potential of L-digestate.

- **L-SSF**

Results are presented in Figure 16.



- M0 and M0_CT gave mass significantly smaller than the other treatments.
- M0.25 CT gave mass significantly higher than M0.5.
- M0 and M0_CT gave diameters significantly smaller than M0.5, M0.5_CT and M0.25.
- M0.25_CT gave diameter significantly higher than M0.
- M0.5_CT gave diameter significantly higher than M0.5.

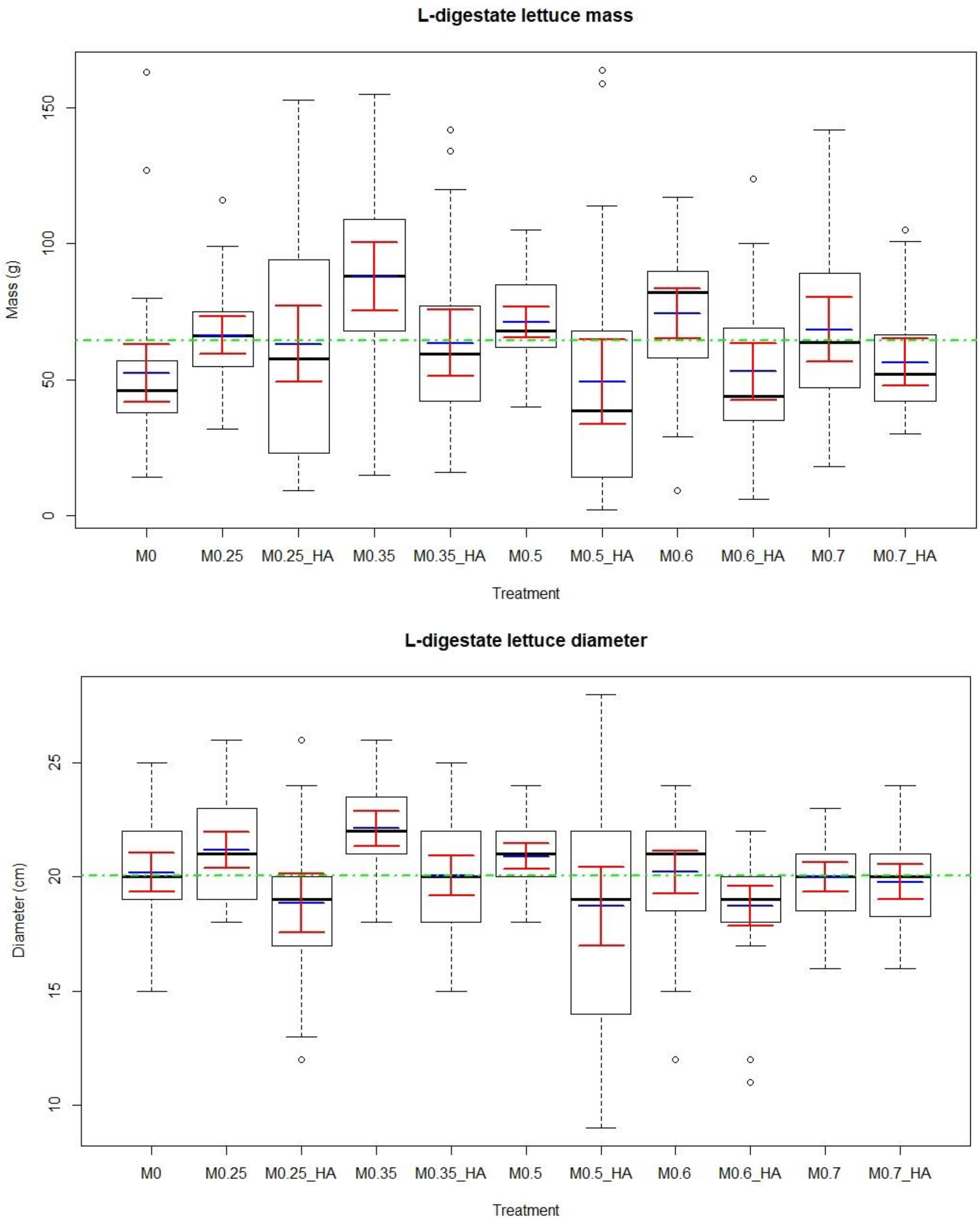
Figure 16: Trial 2 – L-SSF - lettuces mass and diameter

For L-SSF the two treatments without molasses gave mass of lettuces (mean 6 g) significantly smaller than all the other treatments, and diameter (mean 9 cm) significantly smaller than three other treatments. It seems that molasses added to L-SSF had a positive effect on the mass of lettuces. L-SSF mass and diameters were smaller than for L-digestate.

Precautions need to be taken concerning L-SSF results. Indeed, we had a lack of L-SSF supply during the trial. Because of the volume needed, it appeared complicated to study L-SSF and it was put aside for Trial 3.

4.3.3. Trial 3: L-digestate compost tea, molasses and humic acid

Results are presented in Figure 17.



- M0.35 gave mass significantly higher than M0, M0.5_HA, M0.6_HA and M0.7_HA.
- M0.6 gave mass significantly higher than M0.
- M0.35 gave diameter significantly higher than M0.25_HA, M0.6_HA, M0.7 and M0.7_HA.

Figure 17: Trial 3 – L-digestate - lettuces mass and diameter

In Trial 3, the treatment containing molasses at a concentration of 0.35 ml/l and no humic acid gave mass (mean 88 g) diameters (mean 22 cm) significantly higher than four other treatments. Humic acid seemed to have negative effects on L-digestate lettuces' mass.

4.3.4. Discussion

- **EC and fungi**

In Trial 1 L-digestate results showed clearly that adding F2 at EC 1.2 mS/cm gave the best mass results and it was kept for the following trials. For the other EC modalities with L-digestate, F2 had either no effect or positive effect on the mass of lettuces, depending on the EC modality. F1 did not have effect on the mass. Concerning diameter, both F1 and F2 combined with L-digestate did not have effect. A supposition is that the quantity of the products added could have been too low to see any difference.

- **L-digestate and L-SSF origin impact**

As both L-digestate and L-SSF came from food waste treated by AD, it was assumed for the trials that they would have similar properties and it was decided to use in Trial 2 on L-SSF the same treatment of EC and fungi as for L-digestate (F2 at EC 1.2 mS/cm). However, L-SSF does not come from L-digestate but is an extraction of S-digestate. As L-digestate and S-digestate have different chemical composition, it is likely that L-SSF and L-digestate as well. Moreover, L-digestate and L-SSF did not come from the same digestate in this trial, as we had to import it from different locations. For the future it would be needed to determine more precisely the best EC and fungi for L-SSF.
Moreover, different L-digestate sources were used for each trial, coming from different providers, which could have created a bias from a trial to another.

- **Compost tea and molasses on L-digestate**

On L-digestate, it was concluded that the optimal molasses concentration was 0.35 ml/l to get the highest mass of lettuces. Compost tea did not have significant effect though average mass was higher at equal molasses concentration.

- **Compost tea quality**

These results raised more questions on the quality of the compost tea used. Indeed, as seen in the state-of-the-art, compost tea has numerous properties that have been highlighted by several studies, and particularly by Ingham (2005). One hypothesis is that compost tea quality is highly related to where the compost comes from and its components. Then, compost tea composition can change and react differently in function of the season and temperatures. Also, the compost tea should be used immediately after extraction, since the storage reduces the microbial population (Islam *et al.*, 2016). However, even if the compost tea was reoxygenated during 24 to 48h before use, it was transported without oxygenation for 1 h. In addition, compost tea quality also highly depends on the process used (Ingham, 2005; Islam *et al.,* 2016).

- **Molasses on L-SSF**

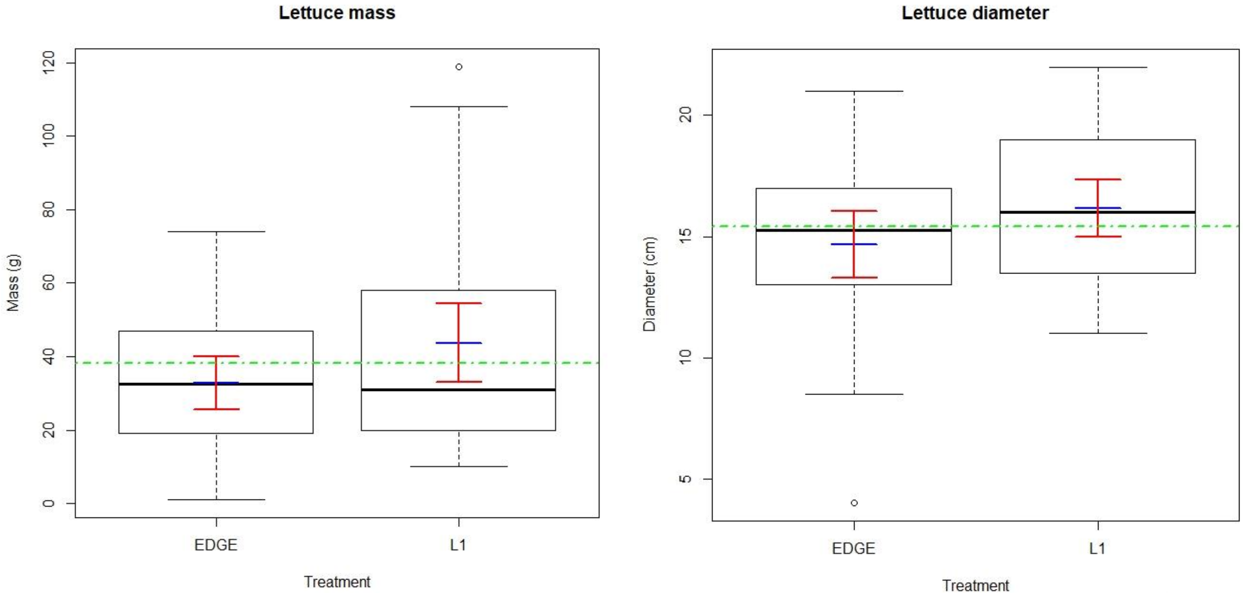
On L-SSF, molasses had a positive impact on mass of lettuces compared to the treatments without molasses. This result needs to be qualified regarding the context. L-SSF was available only during the two first week of the trial. Indeed, important volumes of L-SSF were needed, but it was not possible to provide them on time during the trial. Thus, lettuces of L-SSF treatment did not have fertilizer from the third week. Even if not usually used as only a fertilizer, molasses contains nutrients, which could explain the worst results with low or no molasses concentration. Thus, the conclusions made for the L-SSF in Trial 2 are consistent only in this context of global nutritive deficiency.

4.4. EDGE EFFECT

Edge lines and their neighbours were compared, considering that both had the same treatment. The objective was to determine if edges are more sensitive to wind and temperatures than other lines more protected from these conditions. P-values are not indicated, for additional information about the p-values, please see the Annex 1, where they are all referred.

4.4.1. Trial 1, Edge 1

Edge 1 was compared with its neighbour, Line 1. Results are presented in Figure 18.



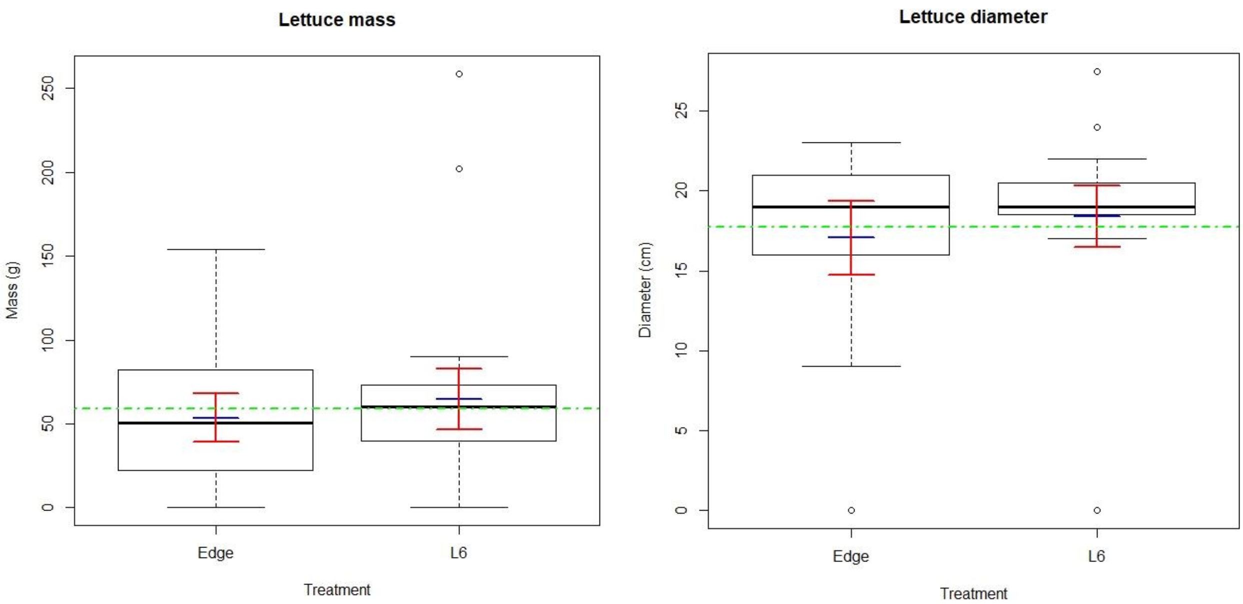
- There was no significant difference between mass and diameters.

Figure 18: Trial 1- Edge 1 - lettuces mass and diameter

Ho was accepted. However, it was very close to the threshold for marginal significance. There is a weak trend (for both mass and diameter) showing lower amounts of lettuce close to the edge. There is a small edge effect for both mass and diameter.

4.4.2. Trial 1, Edge 2

Edge 2 was compared with its neighbour, Line 6. Results are presented in Figure 19. Edge 1 and Edge 2 test were not carried out together as they did not receive the same treatment.



- There was no significant difference between mass and diameters.

Figure 19: Trial 1 - Edge 2- lettuces mass and diameter

There was no edge effect for both mass and diameter.

There was a weak edge effect on edge E1 (west of the greenhouse) and no edge effect on edge E2

(east of the greenhouse) caused by different conditions due to location. The edge line E1 was closer to the door of the greenhouse, thus more in contact with wind and temperature variation. The edge E2 was in the centre of the greenhouse, further from the door. Moreover, the edge 2 side (east) was less exposed to wind as closer to trees.

4.5. GENERAL DISCUSSION

4.5.1. EC and pH management

EC was difficult to manage because it changed quickly in function of the stage of development of lettuces, temperatures and microorganisms' activity. At the beginning, fertilization was managed only looking at the EC and then it was coupled with field observations of nutrient deficiencies. It was especially challenging for organic fertilizers and bioproducts from AD and SSF.

pH was not controlled for organic fertilizers and bioproducts from AD and SSF. Values tended to vary from a fertilizer to another. Indeed, Po B solution had most of the time a pH between 7.0 and 7.5, when L-digestate solution had a pH between 7.5 and 8.5. High pH values measured in L-digestate solution could be caused by the high pH value of the stock solution.

4.5.2. Clogging fertilizers

Organic fertilizers and bioproducts from AD and SSF contained more impurities compared to mineral solutions. The residues blocked frequently the capillarity and thus the irrigation of the towers. When happening, capillaries were cleaned of residues. However, some lettuces were in lack of water during few hours, creating a delay of growth compared to the other lettuces.

5. Conclusion and Outlook

5.1. CONCLUSION

An overview of the Liquid fertilizers field test results is presented in Figure 20.

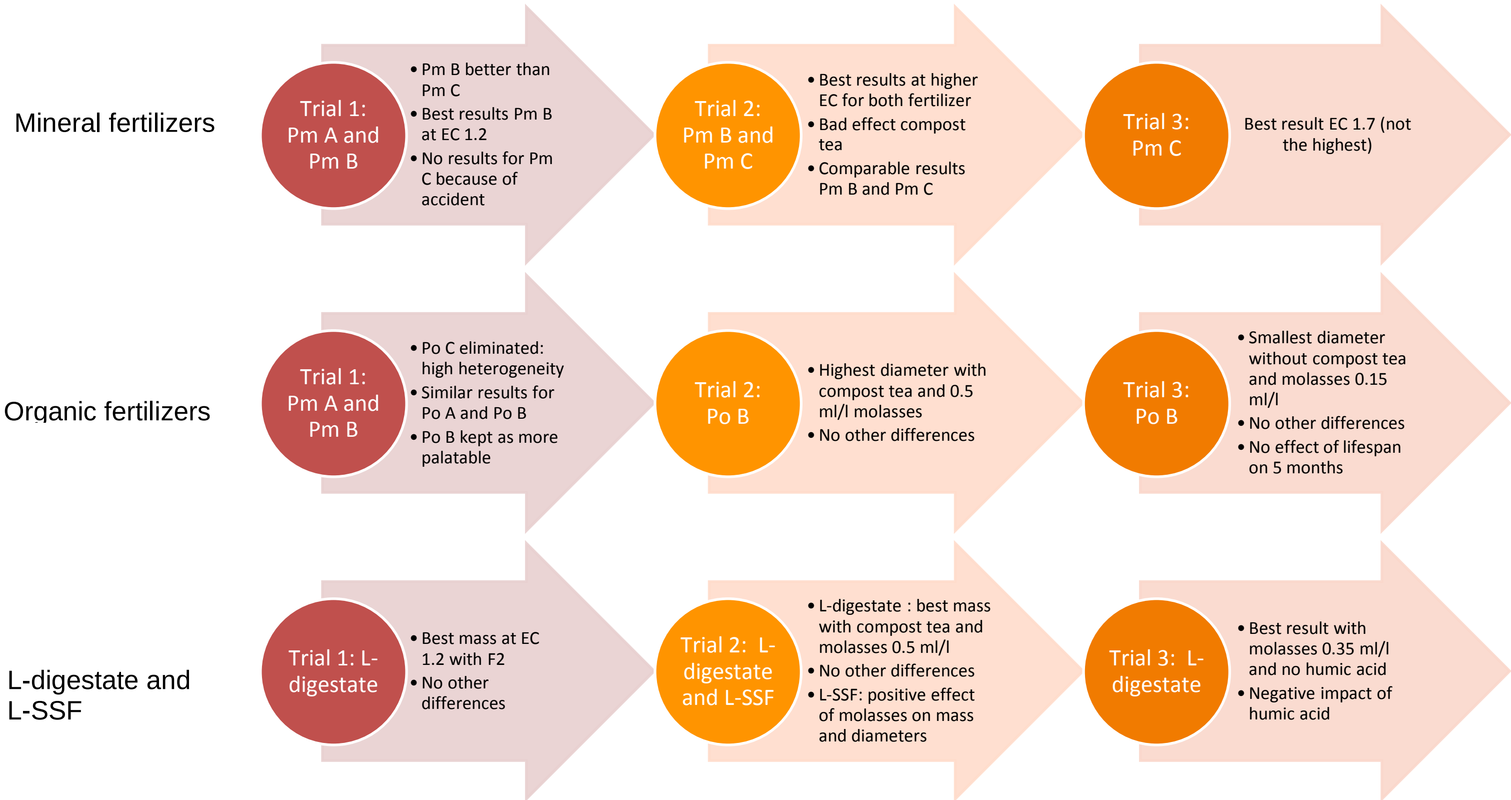


Figure 20 : Overview of Liquid fertilizers field test results on the three categories in the three trials

For mineral fertilizers, three products were tested at different EC and with or without compost tea. Fertilizer Pm C gave the best result at EC 1.7 mS/cm without compost tea.

For organic fertilizers, three products were tested at different EC, with two different fungal products, with or without compost tea and at different molasses concentrations. Po B and Po A gave positive and comparable results and Po B was considered as more interesting as more palatable to consumers than Po A. Po C fertilizer was not considered efficient because of its high heterogeneity in mass and diameters of lettuces. Fungal products, compost tea and molasses did not have important effect on mass and diameter of lettuces.

L-digestate was tested at different EC, with different fungal products, with and without compost tea and humic acid. It gave good results with the fungal product F2 at EC 1.2 mS/cm. Compost tea had no effect or a beneficial effect on it and humic acid had no effect or negative effect on it. Molasses were efficient at the concentration of 0.35 ml/l. L-SSF could not be studied properly because of the insufficient volume available when the trials were done.

An efficient mineral fertilizer and an efficient organic fertilizer were identified, as well as EC and biostimulants for L-digestate management. However, the best EC and biostimulants for each of the three fertilizer categories need further investigation. Thus, Trial 4 is currently running, and other trials will be run in Season 2019 to better define optimal EC and biostimulants for each of the three categories.

5.2. TRIAL 4 AND SEASON 2019 FOR PRECISIONS

In order to confirm some statistical results, Trial 4 of Season 2018 is currently running. However, results cannot be included in this report as data have not been collected yet. Trial 4 objectives are to validate the best mineral fertilizer at its best EC, the best organic fertilizer at its best EC and the best EC and molasses concentration for L-digestate.

Season 2019 will begin in spring 2019. In Season 2019, L-digestate and L-SSF will be produced on site from Lyon or Spain pilot plant, from the AD treatment of food waste collected from restaurants, using a micro-scale AD unit and a SSF unit.

5.3. PROTOCOL IMPROVEMENT

Several improvements will be undertaken:

- A filtration system will be installed to avoid clogging on organic solutions.
- Compost tea management will be improved. Either a different compost tea technique will be identified, or compost tea will be processed onsite to avoid transport.
- Higher EC will be tested on digestate.
- For mineral solution combined with compost tea a better technique of comparison could be used. Such improved technique should consist in measuring fertilizer addition by quantity and not by EC so that compost tea EC would not influence results.
- If appropriate, Po B opened up to 5 months before will be reused as we saw there were no differences caused by lifespan of this length.
- No biostimulants might be used for Po B as we realized that it was already well balanced and not significantly influenced by biostimulants additions.
- Higher fungal product quantities will be tested.
- No more edge lines will be installed on the east side of the greenhouse.

5.4. FURTHER TRIALS

The objective of Season 2019 will be to compare the fertilizing potential of L-digestate and L-SSF with the best mineral fertilizer and best organic fertilizer at their best EC and biostimulants formulations. During Season 2019, two set-ups will be used. The current one will carry-on defining the best protocol for mineral, organic and bioproducts from AD and SSF. Another set-up with more replicates and a lower number of treatments will compare at production scale the identified formulations of mineral fertilizer, organic fertilizer and bioproducts from AD and SSF. Higher number replicates will be carried out to increase the statistical power of the comparison.

In 2019 a bigger reactor to produce L-SSF and S-SSF through SSF process will be installed on Barcelona pilot plant. Thus, L-SSF will be imported for test. Its best EC and fungi products will be studied as it is supposed to be different from the L-digestate ones.

In addition, different analyses will be run in Season 2019 to better define the quality of the plants obtained, regarding taste, heavy metals content, chemical composition and contaminations. The EC will be stablished by the nitrate content even if for the lettuces best results in terms of diameter and weight correspond to higher EC values. Thus, nitrate content will be analysed for all EC to determine the threshold. As heavy metals represent an important consideration in urban farming, analyses will be operated on plants and amendments. Agronomic analyses will be studied in order to have better

understanding of the fertilizers used. In particular, the L-digestate and L-SSF will be tested, to define their quality as fertilizers. For now, quality of the treatments was studied regarding only yield of plants, however, other important factors to define the quality are taste and palatability of the product. If allowed by regulations, sensory analyses will be conducted in Season 2019.
The same treatments will be run at two different seasons (spring and summer) to understand better climate and temperature effect, especially on organic solutions.

Eventually, L-digestate from different origins will be tested to evaluate origin impact. L-digestate made in Lyon pilot plant out of local biowaste will be compared with L-digestate from food industry, from livestock manure and from crop residues. In addition, studying the seasonal effect of biowaste on the L-digestate will be interesting. Indeed, meals served in canteens and restaurants depend on the season. Therefore, L-digestate composition and thus quality could also depend on the season. L-digestate from Lyon pilot and Barcelona pilot will be compared as well.

Field test report 2: Solid fertilizers

ABSTRACT

This report analyses the potential of solid fertilizers produced from digestate and through solid-state fermentation of biowaste within DECISIVE project. Fertilizers are tested in wooden containers. Determination of the best structure and composition of the growing media and comparative results are presented here.

Table of contents Solid fertilisers

1. State-of-the-art

1.1. FERTILIZATION MANAGEMENT

1.1.1. Fertilizers and growing media definition

In horticultural container the fertilization depends on the growing substrate chosen. Growing substrate is composed of organic (peat, coco fibres, bark, etc.) and inorganic matters (perlite, pumice stone, sand, etc.). Elements are characterized by their physical and chemical properties.

Most of the commercial growing substrate sold contains a starter fertilizer to help plants acclimate after plantation. It is usually recommended to fertilize again when the first new leaves appear. Some organic substrates, such as compost, can also contain a biologic activity, with micro and macro organisms.

1.1.2. Physical and Chemical properties

The main physical properties of the growing substrate are apparent density, ability of water retention and porosity to air (Sahin *et al.*, 2002), with the goal to reproduce a soil structure in which roots can develop. The chemical characteristics of interest are pH and EC. EC measures the quantity of nutrients in ionic form, available to plants in a solution. Nutrients are available to plants depending on the pH.

1.1.3. Structure

Several structures of growing substrate exist. They can be either a mix containing organic and inorganic matter or innovative structure such as in lasagna beds. Lasagna beds have been experimented by the Pilot Project of Parisian Productive Rooftop (T4P) with the idea to reproduce a soil by putting down layers of low and high rate of mineralization (Grard *et al.*, 2015). They concluded that the structure in lasagna beds had a positive effect on the production compared to mixed structures. Also, they concluded that earthworms and coffee ground with mycelium had either a positive effect or no effect on the production level and increased the water retention of the substrate.

1.2. COMPOST FERTILIZATION

1.2.1. Fertilizing power

Compost is produced by a solid state controlled microbial aerobic fermentation of organic matter and can be used as a biofertilizer. The feedstock organic matter can come from crop residues, animal waste, food waste, organic municipal and industrial waste. Compost amendment used in agriculture affects microbial activities and fosters a rapid return of biological factors of fertility (Pane *et al.*, 2013).

1.2.2. Compost quality

The quality of compost is defined both by agronomic criteria and innocuousness criteria. Agronomic criteria are explained by the concentration in fertilizing elements and innocuousness criteria by the presence of undesirable elements such as heavy metals, pathogenic elements and coarse elements (e.g. plastic, glass). Compost quality is regulated by the Commission Regulation n°889/2008. Especially, it is forbidden to use some compost in organic agriculture and compost is authorized under conditions regarding the concentration of heavy metals (ADEME, 2015).

1.2.3. Compost versus digestate

In young compost, nutritive elements are quickly released. However, it can burn plants and, if buried, it can create a nitrogen deficit. The old compost releases slowly the nutritive elements without risks for plants.

Compared to compost, digestate presents different physicochemical characteristics, with a higher concentration in fertilizing elements (ADEME, 2015).

2. Objectives

During Season 2018, three trials corresponding to three combinations of soil structure/composition were implemented with S-digestate: “Lasagna bed”, “Fertilizer mix” and “Substrate”. The objective was to assess which structure was the most productive for digestate and to compare it to composts and controls.

3. Materials and methods

3.1. CROPS

To explore the potential of soil, where nutrients are available in a longer period in wood containers compared to hydroponics, plants with longer growing cycle and deeper roots than lettuces were selected: tomatoes (fruiting crop, roots 30 cm deep) and basil (leafy crop, usual companion of tomato and deep roots). Long roots improve growing substrate physical properties and exploit better its fertilizing power.
Plants of each crop were bought to a seedlings' grower. Each plant came from the same growing batch (planted the same day and grown together) and 10 % more seedlings than needed were grown to select the most homogeneous for the trials at plantation.
Tomatoes were cherry tomatoes of the variety *Lycopersicon esculentum* and named “Délice du Jardinier”. Basils were from the variety *Ocimum basilicum*, named “Grand Vert”.

3.2. PRODUCTS

In total, six products were tested. The origin of each of them is described in Table 6:

Table 6: List of the products studied in Solid fertilizers field test

Product	Origin	Description
S-digestate	Partner Suez	From biowaste
Young compost	Neighbor farmer	From biowaste and chopped wood, 6-9 months
Old compost	Neighbor farmer	From biowaste and chopped wood, 9-18 months
Green compost	Neighbor garden center	From green waste
Blond peat	Commercial	With a light fertilizer starter
Horticultural soil	Commercial	Without peat

3.3. WOOD CONTAINER SET-UP

All plants were grown in impermeable wood containers, as shown in Figure 21. They had a tube on their bottom, to access water level or to drain water if needed.
Each wood container was in a group of three (T configuration as in Figure 21). Here a treatment concerns the specific structure and components of a wood container. There were 10 treatments with 3 replicates (i.e. 30 wood containers on an east-west axis as shown on Figure 21 and

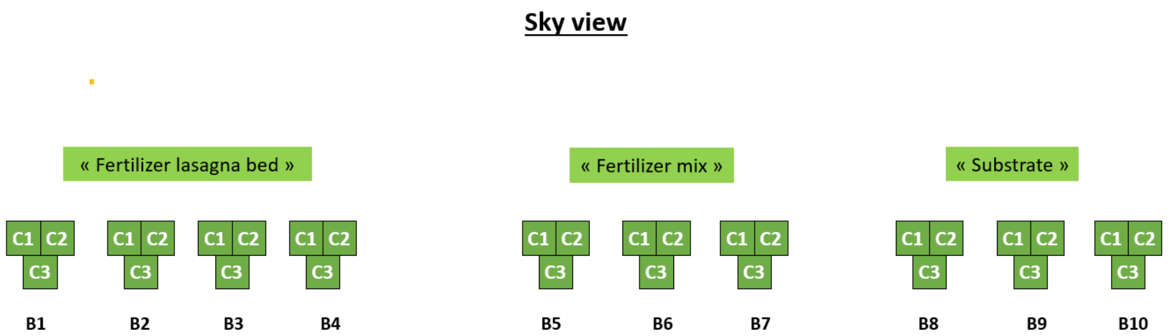


Figure 22).

i: Bloc B7 on the 03.08.2018 ii: Tomato plan on the 09.11.2018

Figure 21: Trial set-up – photo of the wood containers system

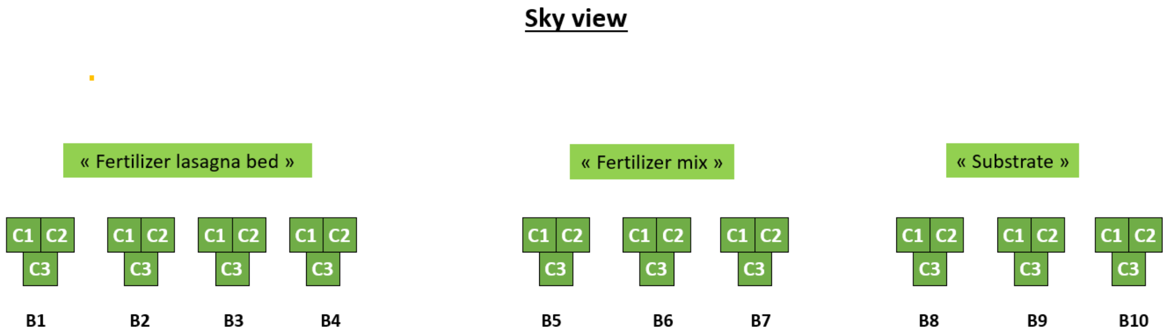


Figure 22: Trial set-up – scheme of the wood containers system

3.4. FIELD TESTS

3.4.1. General features

On the 27th of June 2018, one tomato plant and four basils were planted in each container, as shown on Figure 23. Basil was harvested three times in the summer, with the last harvest on the 13th of September. Tomatoes were harvested along summer and autumn according to their maturity. Finally, on the 6th of December tomato plants were removed.

First, sprinkling irrigation was made by hand. As it was time consuming, a drop by drop irrigation system was installed in August. As soil structure and composition were specific to each bloc and as plants had different growth rate, the irrigation was specific to each container depending on its need for water.

When needed, tomato plants were trained, and their water sprouts removed. In prevention to diseases, aeration and clean up was done regularly. However, in all cases, after identification of a disease, only organic controls were used. In case of aphid attack, black soap was sprayed on the three containers of the concerned bloc and data was recorded of date of spray and identification of the plants. In case of powdery mildew beginning, water was sprayed on all the plants of the container and the concerned leaves removed if needed. If powdery mildew persisted, a sulphur-based solution was sprayed. In case of downy mildew, a copper-based solution was sprayed.

For basils, mass of fresh matter at harvest was collected. Basil was harvested three times in the same day for all the plants. For tomatoes: number of fruits harvested, and their individual mass were recorded. Harvest was done whenever tomatoes were ripe. Ripe tomatoes were characterized by the colour: entirely red. Season 2018 stopped on the 6th December 2018. At this date, the number of all green tomatoes and their mass were recorded as well as the number of left flowers.

Besides, sizes of all tomato plants were measured every week.

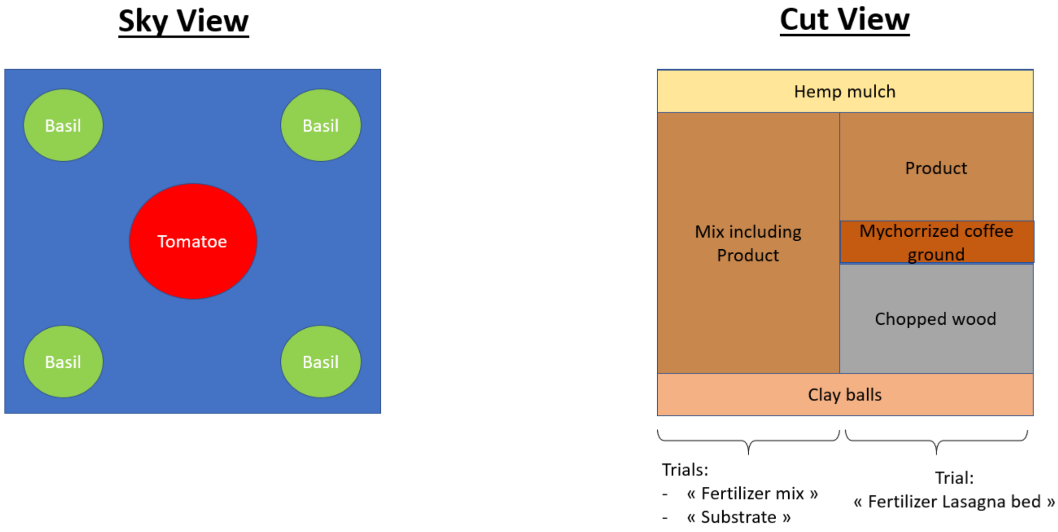


Figure 23: Wood containers crops organisation and structures

3.4.2. Products and composition

For each trial, containers were filled up to their full capacity and clay balls were placed at the bottom to ease drainage. On the top, hemp was installed to retain humidity and avoid weeds as shown on Figure 23.

Three structures were tested. In structure "Lasagna bed", components were organized in lasagna structure. One of the best treatments of T4P (Grard *et al.*, 2015) was reproduced. Above the clay balls, chopped wood was added, then a thin layer of mycorrhized coffee ground and at the top the container was filled with the studied product.

In structure "Fertilizer mix", components were mixed together with 20 % of the container volume of the product and 80 % of blond peat. To assure the homogeneity of replicates, a first general mixture was made and then separated in the different containers.

In structure "Substrate", components were mixed together with 50 % of the container volume of the product and 50 % of soil structuring components with poor or no nutrients. As for Trial "Fertilizer mix", a first general mixture was made and then separated in the different containers.

The experimental design was performed as detailed in Table 3.

Table 7: List of treatments in Solid fertilizers field test

Trial	Abbreviation	Structure	Product
"Lasagna bed"	B1-L-D	Lasagna bed	Digestate
	B2-L-VC	Lasagna bed	Old compost
	B3-L-JC	Lasagna bed	Young compost
	B4-L-DV	Lasagna bed (control)	Green compost
"Fertilizer Mix	B5-F-D	Mix 20 % product, 80 % blond peat	Digestate
	B6-F-VC	Mix 20 % product, 80 % blond peat	Old compost
	B7-F-To	100% blond peat (control)	Blond peat
"Substrate"	B8-S-D	Mix 50 % product, 50 % structuring components	Digestate
	B9-S-VC	Mix 50 % product, 50 % structuring components	Old compost
	B10-S-Te	100 % horticultural soil (control)	Horticultural soil

3.5. STATISTICAL METHODS

- Basil fresh mass comparison

A plant of basil was considered as a replicate. There were 12 replicates per treatment. Statistical methods of analysis were conducted as explained for the hydroponics field test (Paragraph 3.6 page 20).

- Tomato mass

Tomatoes from the same plant were pseudo-replicates. The analysis were done using multiple comparison statistical test as explained for Liquid fertilizers field test (Paragraph 3.6 page 20).

- Number of tomatoes, yield and growth rate comparisons

A tomato plant was considered as a replicate. The multiple statistical tests used were the same as explained for Liquid fertilizers field test. Growth curve were drawn using height of tomato plants from plantation to the end of the trial (Paragraph 3.6 page 20).

In the report, results are visualized using boxplot on which means of each treatment are illustrated in blue, 95 % confidence intervals in red and mean of all treatments in green. Limit of box corresponds to the lower and upper quartiles. Whiskers were defined using the following formulas:

Upper whisker = min (max (x), Q3 + 1.5 * IQR)

Lower whisker = max (min(x), Q1 – 1.5 * IQR)

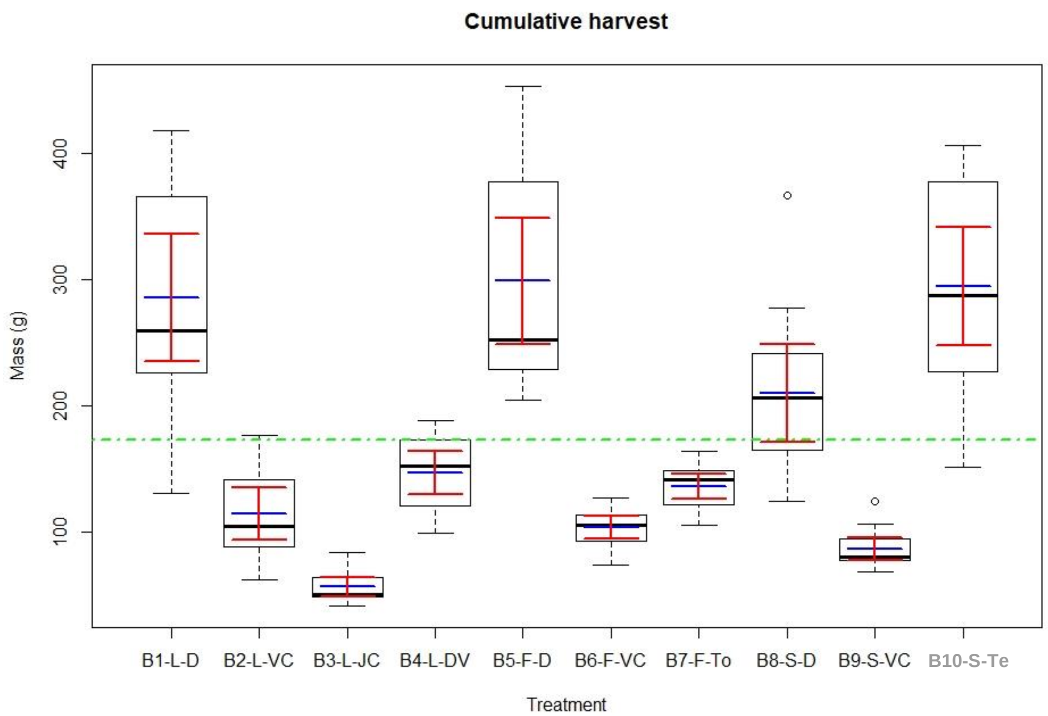
Where Q1 is the lower quartile, Q3 the upper quartile and IQR = Q3-Q1

4. Results

In this section, mass of basil harvested is studied, followed by the tomato harvest. Then, the growth rates of tomato plants are analysed, followed by the discussion. P-values are not always indicated, for additional information about the p-values, please see the Annex 2.

4.1. BASIL: FRESH MASS

Three harvests of basil were made and statistical analysis of the first harvest and of the sum of the three harvests was done. Results for the sum of the three harvests are presented in Figure 24. Photos of the three harvests are available in Annexes 3, 4 and 5.



- “Lasagna bed”: B1 gave mass significantly higher than B2, B3 and B4.
- “Fertilizer mix”: B5 gave mass significantly higher than B6 and B7.

- “Substrate”: B8 and B10 gave mass significantly higher than B9.
- global: B1, B5, B8 and B10 gave mass significantly higher than all the other treatments. Except B8, who gave mass not significantly different from B4.

Figure 24: Cumulative fresh mass of basil harvested for each treatment

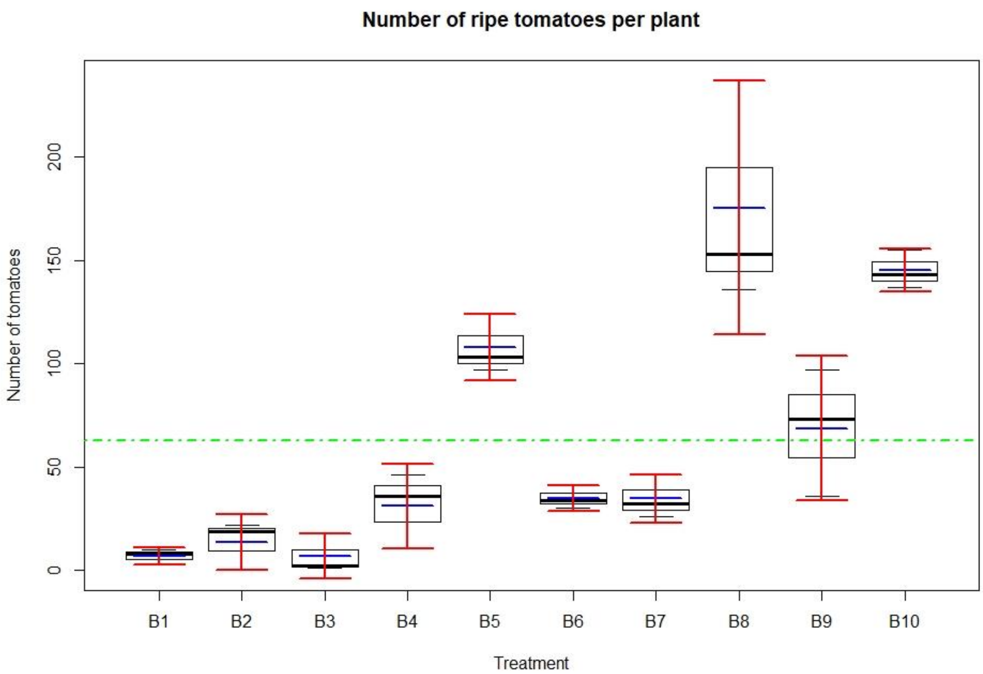
In “Lasagna bed”, S-digestate basils fresh mass (mean 286 g) was significantly higher than the two composts and control. In “Fertilizers mix” S-digestate (mean 299 g) had basils heavier than those from compost and control. In “Substrate”, S-digestate (mean 210 g) was not different from the control and both had significantly heavier basils than the compost.

Comparing the three trials, Horticultural soil (control from “Substrate”) resulted in the biggest mass of basil harvested, followed by the three treatments containing S-digestate in each trial. S-digestate treatments were not significantly different from Horticultural soil.

Besides, statistical results of the first harvest gave similar information, presented in Annex 6.

4.2. TOMATO: NUMBER OF FRUITS

Figure 25 presented the results for the number of ripe tomatoes.



- “Lasagna bed”: no significant difference between B1, B2, B3 and B4.
- “Fertilizer mix”: B5 gave number of tomatoes significantly higher than B6 and B7.
- “Substrate”: B8 and B10 gave number of tomatoes significantly higher than B9.
- global: B8 gave number of tomatoes significantly higher than all other treatments except B10.

B10 gave number of tomatoes significantly higher than all other treatments except B8 and B5

Figure 25: Number of ripe tomatoes per plant for each treatment

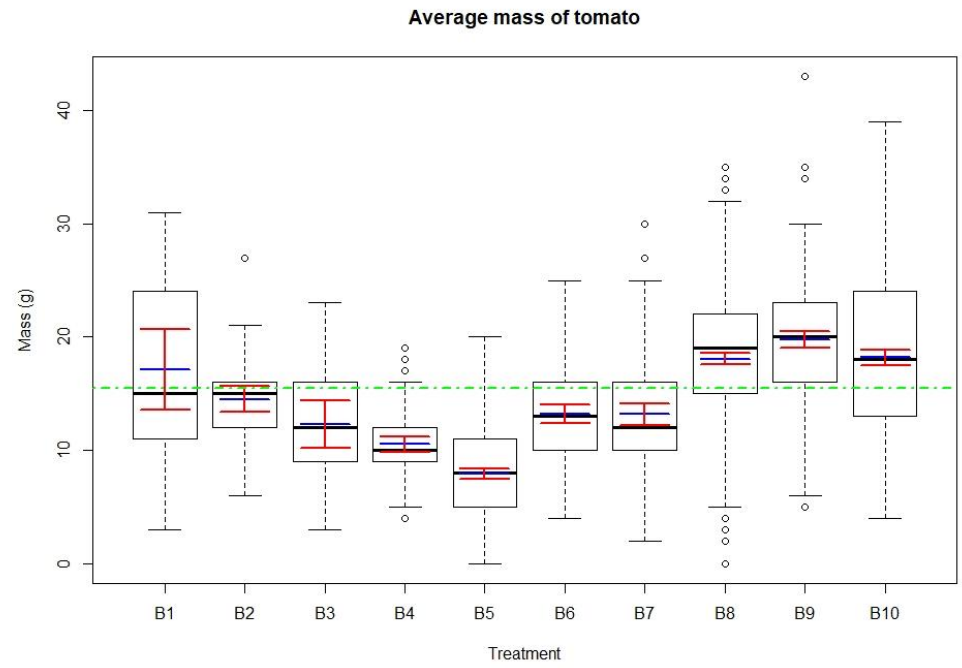
In “Lasagna bed” there were no differences between treatments (mean 7 fruits/plant for S-digestate). In “Fertilizer mix”, more tomatoes grew with S-digestate (mean 100 fruits/plant) than in presence of compost and control. In “Substrate”, S-digestate (mean 175 fruits/plant) was not different from the control and both had significantly more fruits than the compost.

Comparing the three trials, S-digestate and control from “Substrate” both gave more fruits than all the other treatments and were not different from each other.

Adding green tomatoes and flowers led to the same results presented in this section for the ripe tomatoes (see Annex 7).

4.3. TOMATO: MASS OF FRUITS

- Average mass of tomatoes per plant is presented in Figure 26



- **“L**asagna bed”: B1 and B2 gave tomatoes with mass significantly higher than B4.
- “Fertilizer mix”: B5 gave mass of tomatoes significantly lower than B6 and B7.
- “Substrate”: no significant difference between B8, B9 and B10.
- global: B8, B9 and B10 gave mass of tomatoes significantly higher than all the other treatments except B1.

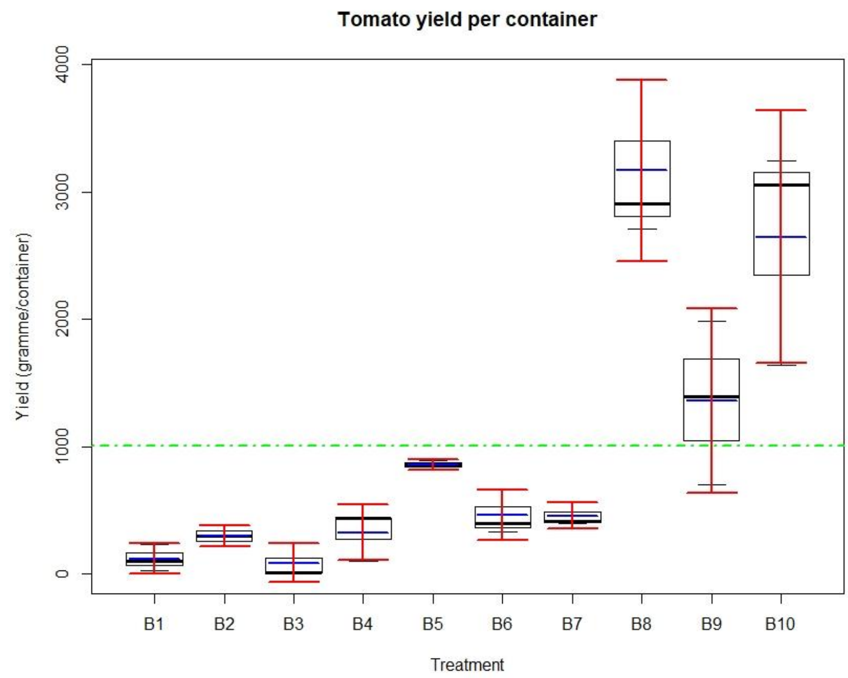
Figure 26: Mass of tomatoes harvested for each treatment

In “Lasagna bed”, S-digestate and old compost resulted in tomatoes with mass significantly higher (mean 15 g) compared to young compost and control. For “Fertilizer mix”, S-digestate produced tomatoes with mass (mean 8 g) significantly lower than the other treatments. In Trial “Substrate” there were no significant differences (mean 18 g for S-digestate).

Comparing the three trials, the three treatments of Trial “Substrate” had tomatoes significantly heavier compared to all treatments except S-digestate from “Lasagna bed”.

4.4. TOMATO: PLANTS YIELD

Average yield of tomato plants is presented in Figure 27.



- “Lasagna bed”: no significant difference between B1, B2, B3 and B4.
- “Fertilizer mix”: no significant difference between B5, B6 and B7.
- “Substrate”: B8 and B10 gave yield higher than B9.
- global: B8 and B10 gave yield significantly higher than all the other treatments.

Figure 27: Yield of tomato per plants per container for each treatment

In “Lasagna bed” (mean 120 g/plant for S-digestate) and “Fertilizer mix” (mean 856 g/plant for S-digestate) there were no differences between treatments. In “Substrate”, S-digestate (mean 3168 g/plant) and control (mean 2646g/plant) had a higher yield than compost (mean 1358g/plant) and were not different.

Comparing the three trials, S-digestate and Horticultural soil (control) from “Substrate” had the best yield of all the treatments and their related values were similar.

4.5. TOMATO: PLANTS GROWTH RATE

Results for the tomato plants growth rates for the three trials are presented in Figure 28.

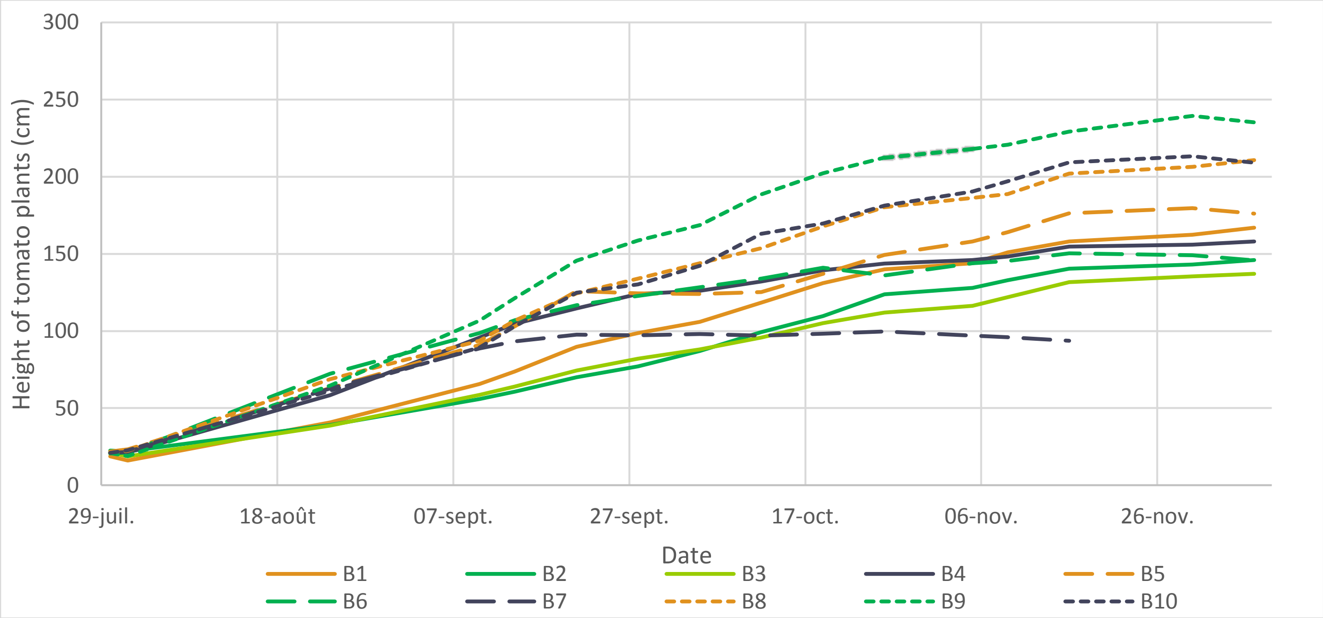
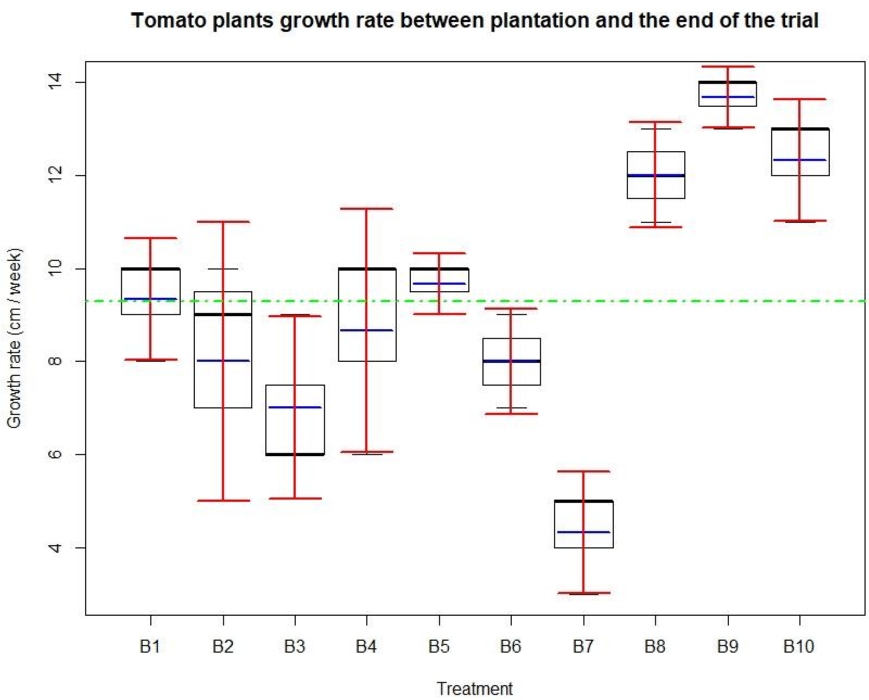


Figure 28: Tomato plants mean growth curves for all the treatments

As shown by Figure 28, on the 30th November Trial "Substrate" (B8, B9 and B10) gave higher tomato plants than Trial "Lasagna bed" and Trial "Fertilizer mix". Compost from Trial "Substrate" (B9), containing compost, had the highest tomato size of the 10 treatments. For Trial "Fertilizer mix", the three tomato plants of B7 died at the end of October. Therefore, it resulted in the smallest size at the end of the trial with less than 100 cm.



- "Lasagna bed": no significant difference between B1, B2, B3 and B4.
- "Fertilizer mix": B5 gave growth rate significantly higher than B7.
- "Substrate": no significant difference between B8, B9 and B10.
- global: B9 gave growth rate significantly higher than B1, B2, B3, B4, B6 and B7.

Figure 29: Tomato plants growth rates between plantation and the end of the trial for all the treatments

In "Lasagna bed" and "Substrate", there were no differences between treatments. In "Fertilizer mix", S-digestate had a growth rate (mean 10 cm/week) significantly higher than the control (mean 4 cm/week). It must be considered that tomato plants on the control died before all the other plants.

Comparing the three trials at the end of the experiment, the plants of "Substrate" were taller (mean 13 cm/week) than the plants from the other trials (i.e. "Lasagna bed" and "Fertilizer mix")

4.6. DISCUSSION

4.6.1. Yield

Maximum yield obtained for the tomatoes was 3886 g/container in the Trial "Substrate" containing digestate. As a comparison, T4P obtained a maximum yield around 3000 g/box in 2012 and 3500 g/box in 2013 (Grard *et al.*, 2015). It was obtained in the configuration lasagna with a structure similar to our experiment "Lasagna bed".

4.6.2. Lasagna bed structure

In T4P trial on Paris rooftop, Grard *et al.* (2015) obtained that putting the different substrates in layers instead of mixing them had a positive effect on the yield of lettuces and tomatoes. However, a maximum yield of 439 g/container was obtained in the trial of this report for the lasagna configuration while T4P obtained 3500 g/box. With the information studied, it is supposed that the lasagna bed presented in this report had the same proportion of products than the lasagna used by T4P. It needs to be considered that in T4P, four plants of tomatoes were grown per container while there were only one plant per container in the present Solid fertilizers field test. Moreover, T4P was outside on a rooftop and DECISIVE Solid fertilizers field test was in a greenhouse. Nevertheless, it is supposed that the structure lasagna beds could show its complete potential on longer terms studies more than on one growing cycle only, due to the time needed for the different beds to evolve. Besides, properties of used products can have been different. Moreover, quantity of product (digestate and compost) was different between trials as explained below.

4.6.3. Quantity of product

Trial "Substrate" gave the best yield. However, the quantity of products (digestate and compost, 50 %) was higher in this trial compared to the others (41 % "Lasagna beds" and 20 % "Fertilizer mix"). That could explain the better development of tomato plants on this growing substrate. However, this difference did not appear for basil yield (fresh mass harvested). This could be explained by the fact that this crop grew first and got all required nutrients. However, tomato plants grew during a much longer time; thus, they could have felt the lack of nutrients in some treatments and were able to show the differences in the three trials.

4.6.4. Competition between basils and tomato plants

There could have been competition for nutrients and sunlight between the two crops. Before second basil harvest, some basils shade tomato plants as it can be seen on pictures in Annex 4. However, at the third harvest of basil, tomato plants of some treatments were well developed: they were shading basils, thus decreasing their developments. These differences can be seen in the pictures of Annex 5.

4.6.5. Climate condition

All blocs were facing south, however, the bloc B1 of the trial was located close to the west door of the greenhouse, when the bloc B10 was in the centre of the greenhouse. First blocs, close to the door, were exposed to more wind and colder temperature when the door was open. The other plants were less exposed. Thus, from B1 to B10, there was a gradient of exposition to the wind and colder temperatures. The plants in Trial "Substrate" could have partly grown better due to more favourable temperature and wind protection, while Trial "Lasagna bed" was more exposed to these conditions.

4.6.6. Irrigation management

Irrigation management proved more challenging than expected. Irrigation in containers is usually made using two characteristics linked to each substrate: irrigation dose and frequency. Irrigation dose is supposed to be the same each time, while frequency depends on a lot of climate and crop conditions and needs a lot of observation. Due to their different configurations, each bloc had different irrigation doses and frequencies.
We had high temperature during August, and some plants suffered from hydric stress caused by a late

identification of hydric stress. When pointed out, irrigation management was modified in order to adjust the water need to higher frequency and less quantity. All blocs were concerned, but the Trial “Fertilizer mix” could have been the most impacted. Indeed, this trial was composed of blond peat mixed with the studied products. However, even if blond peat is a very interesting substrate as it retains water, when dry it becomes hydrophobic. Thus, this trial could have suffered even more than the others from the hydric stress.

4.6.7. Compost quality

S-digestate gave higher number of tomatoes than compost made from food waste and green waste. The providers of the compost (neighbour farmers) were using it mostly as a structuring element for their field and the compost was woody. A supposition is that the ratio carbon to nitrogen was high (wood chip has a ratio of 500-650 - Martin, 2015). The ratio carbon to nitrogen is an indicator of the humic potential of the compost. Higher is the ratio, slower is the degradation of the organic matter in the soil. However, the nitrogen contained in the wood chips is slowly degraded, and so slowly available to plant. In the field test, a young compost would have had a lower ratio carbon to nitrogen (15), and elements releasing quickly nitrogen to the soil. This compost would have acted as a fertilizer and would have stimulated the microbiological activity of the soil (AGRIBIOVAR and Chambre de l’agriculture du Var).

4.6.8. S-digestate and plastic

The S-digestate contains pieces of plastics due to the grinding of plastics bags used to collect food waste. On one hand, it might bring porosity to the soil. On another hand, the plants developed roots in contact with plastic. Pieces of plastics are macroscopic and not microscopic size. Several subjects could be dig in to understand which kind of plastic it was present, if the plastic breaks down during the growth of plants, if the roots can absorb plastic and if a contamination to plastic can occur. In Lyon pilot plant, the objective is to avoid having plastic in the treated biowaste. Thus, such study won’t be needed.

4.6.9. Tillage

No tillage was operated in the containers during the trial as lasagne bed should not need it and the protocol aimed to not add variable between blocs. When weeds grew, they were removed by hand. However, the tillage could have helped with the aeration of the soil, especially for Trial “Fertilizer mix” and Trial “Lasagna beds” where soils were the most compacted at the end of the trials, in December. Some containers had lost up to 8 cm height. Adding more substrate to complete this compaction could have benefited the plants.

4.6.10. Strength of statistical results

Due to space and time constraints, each treatment was repeated only in three containers, making twelve replicates per treatment for the basil analysis but only three replicates per treatment for the tomato plants. Thus, regarding the results it must be considered that these small numbers of replicates directly impacted the power of the statistical analysis.

5. Conclusion and outlook

5.1. CONCLUSION

An overview of the Solid fertilizers field test results is presented in Figure 30 and Figure 31.

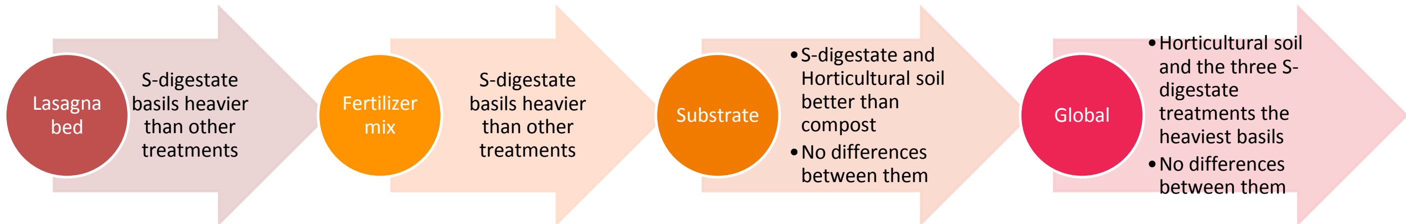


Figure 30 : Overview of Solid fertilizers field test results on basil

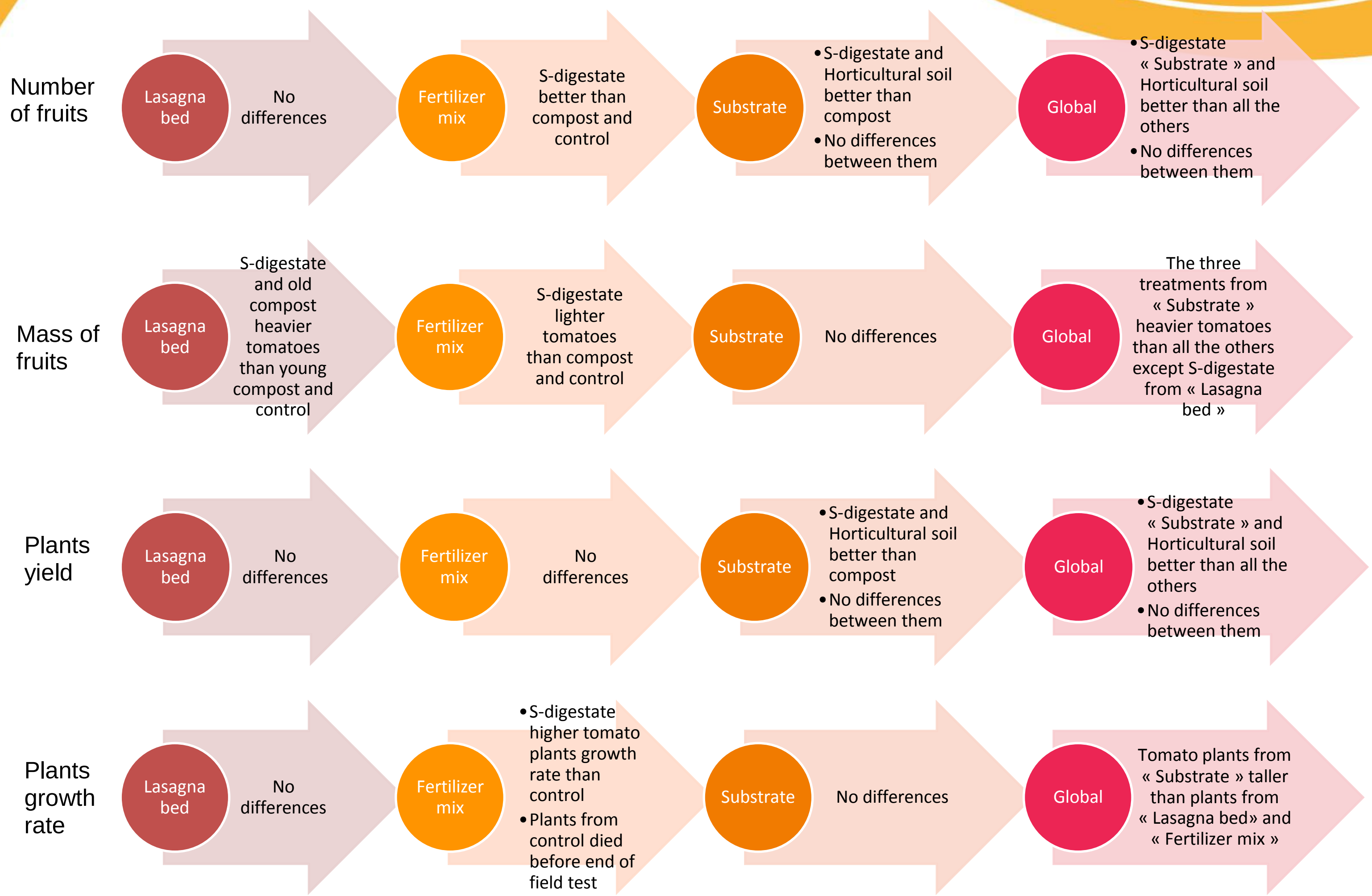


Figure 31 : Overview of Solid fertilizers field test results on tomato

S-digestate proved to be an efficient fertilizer. S-digestate from “Lasagna bed” and “Fertilizer mix” along with Horticultural soil gave the highest fresh mass of basil. S-digestate from “Substrate” and “Fertilizer mix” along with Horticultural soil gave number of tomatoes higher than the other treatments.

“Substrate” structure proved to be efficient. Its three treatments gave an average mass of tomatoes and growth rates higher compared to the other trials. Besides S-digestate and Horticultural soil from this structure had the best tomato yield and Compost resulted in highest growth rates.

Conclusion is that structure “Substrate” with 50 % S-digestate mixed with 50 % structuring component was the most efficient configuration to use digestate in order to have the highest number and mass of tomatoes, and so the highest yield. The two other configurations were more efficient for basil fresh mass.

Therefore, Trial “Substrate” seems to be the most interesting to study in Season 2019. However, the origin of the product and protocol introduced some bias with different quantities of products between trials, challenging irrigation management and poorly efficient compost. Protocol and product quality need to be improved to have more useable results.

5.2. PROTOCOL IMPROVEMENT

Several improvements will be done:

- To avoid too much difference of climate conditions between treatments, blocs will be moved away from the entrance and centred in the middle of the greenhouse.
- To improve number of replicates, there will be one more container per bloc, so four per bloc.

- Moreover, irrigation management will be improved. Doses will be calculated for each bloc in function of the substrate properties and plant needs. Then, the same dose will be applied each time. Frequency will be managed directly by a more careful observation of the blocs including checking soil moistening. Besides, a soil structure specialist will be consulted.
- Different analyses will be run to better define the properties of the products inputs, growing media and plants as well as growing media evolution. A focus will be made on taste, heavy metals, plastic contamination, chemical composition, physical properties and contaminations. Concerning taste analysis, regulation regarding trial products needs to be further studied.
- Compost with a lower ratio carbon to nitrogen and a lower quantity of chips wood will be used in Season 2019 in order to have a more efficient comparison with digestate. The ratio value still needs to be determined in function of the composition and the month it will be installed.
- In Season 2019, trials could be performed using the same quantity of product whether it is lasagne bed, peat mix or structuring mix.
- Trial will be run longer in order to study product evolution in time. Thus, it should start in spring 2019 and end in summer 2020 allowing crop rotation: basil-tomatoes, then lettuce, then green manure and back to basil-tomatoes. With a two years study, Trial “Lasagna bed” could be studied, as it should give better results than in a one-year study.
- Besides, product impact on short root crop (lettuce) will be tested as well as its reaction to green manure.
- Tillage and addition of complementary growing substrate to avoid compaction will be considered.

5.3. FURTHER TRIALS

In Season 2019, the fertilizing power of S-Digestate and S-SSF from the same digestate origin will be compared. According to the timing and the SSF reactor location, these products will be produced on the Lyon demonstration site or imported from the Spain demonstration site.

Digestate origin could be studied as well, with the comparison of different digestates from similar food waste, or even the comparison of different digestates from different waste origins like manure. Besides, impact of seasonality on digestate fertilizing power could be studied.

Eventually, an open field test could be performed with the same digestate and compost as those used in wood containers in the greenhouse.

Field test report 3: Biopesticide

ABSTRACT

This report analyses the potential of biopesticides produced from digestate through solid-state fermentation of biowaste within DECISIVE project. *Bacillus thuringiensis* from the solid-state fermentation is used on the pest *Pieris brassicae* and compared to a commercial product. First trials and comparative results are presented here. Further trials are planned for spring-summer 2019.

Table of contents Biopesticide

1. State-of-the-art

1.1. THE SECTOR OF BIOCONTROL

1.1.1. The biocontrol definition

“Biocontrol” term is used both for biocontrol products and for methods of organic crops protection. Biocontrol products are defined as “agent and products using natural mechanisms as part of integrated pest management programs against crops enemies” (Loi d’avenir pour l’agriculture l’alimentation et la forêt, 2014). It includes crop protections products like microorganisms, chemical mediators and natural substances from plants, animals or minerals (Article L253-6, code rural). In addition, from the European Biostimulant Industry Council (EBIC), biocontrol methods refer to the use of auxiliary insects, biological insecticides, and natural substances to protect crops against harmful organisms (EBIC, 2018).

1.1.2. European regulatory framework: biocontrol products

In 2009, European Union wrote the Directive for the Sustainable Use of Pesticide (Directive 2009/128/EC), aiming to reach a sustainable use of pesticides through a European framework. The objectives are to reduce the impact of pesticides on human health and environment and to incite the use of integrated methods in alternative to chemical products. Biocontrol products are not concerned by this text for now. In France, the biocontrol market is 140 M€ (2017), corresponding to 5 % of the crop protection market, with an increase of the activity of 25 % between 2016 and 2017. The market corresponds at 57 % to natural substances, 29 % to microorganisms, 18 % to chemical mediators and 12 % to macro-organisms (IBMA France, 2017).

1.2. *BACILLUS THURINGIENSIS* USED AS A BIOPESTICIDE

Bacillus thuringiensis (Bt) was the first microorganism to be homologated in the world as a biopesticide, in the US in the 1960’s and in France in 1970’s. It is now the most used microorganism in biocontrol. *Bt* is a gram-positive *bacillus* synthesizing a parasporal crystal protein during sporulation. This crystal contains protoxins, which have a larvicide activity on different insect species from the following orders: lepidoptera, coleoptera and diptera (Höfte and Whiteley, 1989, Sacchi *et al.*, 1986). The *Bt* crystals dissolve in the larval midgut and are proteolytically converted into smaller toxic polypeptides. This activated toxin interacts with midgut epithelium cell of insects leading to the cells swell and lyses (Höfte and Whiteley, 1989). Therefore, the larvae stop feeding and die.
Commercial formulations of *Bt* are used as biopesticide to control agricultural pests, proposing a powerful alternative strategy for protection of crops against insect damages (Höfte and Whiteley, 1989).

1.3. *BT* GROWN THROUGH SSF WITH DIGESTATE AS THE FEEDSTOCK

SSF studies demonstrate the feasibility to produce *Bt* based biopesticide with more than 10^{10} CFU/g DM viable cells obtained using wastewater sludge or its mixture with agricultural waste (Zhuang *et al*, 2010). In addition, a study showed the feasibility to use kitchen waste to produce biopesticide *Bt* by SSF (Zhang *et al*, 2013). Also, Ballardo *et al.* (2015) demonstrated the possibility to use soy fibre residue valorised as organic soil amendment with biopesticide properties though aerobic SSF in presence of *Bt*.
Research performed by UAB, partners in the project DECISIVE, showed good results in terms of the growth of *Bt* using SSF process with digestate as the feedstock. The microorganisms were able to colonize the entire matrix grow and sporulate in hygienized (1 h at 70 ºC) solid digestate. They reached a maximum spore production of $2.85\pm0.22\ 10^7$ CFU/g DM (Cerda *et al.*, 2018).
Based on this context, Biopesticide field test was performed to study the biopesticide effect of *Bt* from L-SSF on *Pieris brassicae*, a pest of cabbages.

2. Objectives

During Season 2018, two trials were conducted on *Pieris brassicae* caterpillars living on cabbages in containers, and one trial on the caterpillars living in small trays containing cabbage leaves. The objective was to assess L-SSF biopesticide performance by comparing it with a commercial *Bt* solution.

3. Materials and methods

3.1. PEST AND CROP

Pest tested was *Pieris brassicae* (Large White butterfly), a lepidoptera whose caterpillars feed with cabbage leaves. Caterpillars go through stage L1 to L5 before transforming into chrysalis. As *Bt* affects young caterpillars only, it was chosen to use caterpillars of stage L2-L3, depending on the provider stocks. Individuals at stage L1 could not be used as they are not resilient enough to be handled.
Cabbages used were cauliflower, variety Belot. Each was planted in a horticultural container of 5 l or 3 l (depending on the trial) and grown in a horticultural fertilizer soil.

3.2. PRODUCTS

Products used were L-SSF and Commercial *Bt* solution.
L-SSF was provided by UAB. The solution contained *Bt var. kurstaki* at a *Bt* concentration of 10^8 CFU/ml. The solution was stored in the fridge and 24h before use, removed and stored at room temperature. For each trial, fresh L-SSF was sent by UAB.
Commercial *Bt* solution was also *var. kurstaki*. The form was a water-soluble product from Solabio (Delfin jardin) at 32.10^6 UIAK/g. Based on Solabio recommendations for cabbage crops the solution was used at a concentration of product of 1 g/l. According to manufacturer’s instructions, the product should be applied when the first larvae appear or 3 days before harvest. Application can be renewed every 6 to 14 days with a maximum of 3 applications per generation (APREL *et al.*, 2017).
Control had nothing sprayed on.

3.3. SYSTEM SET-UP

The containers for the trials were installed on a large elevated tray in a greenhouse. Due to the presence of lizards in the greenhouse, and to avoid caterpillars moving away from the trials or between group of cabbages, one net per treatment was installed around each group of cabbages receiving the same product spraying. Cabbages were irrigated at the collar when needed.
Small trays trials were installed on shelves in a shed at office temperature. Caterpillars were fed by adding leaves of cabbage each time it was needed.

Figure 32: Biopesticide field test experimental set-up - Cabbage containers and trays

3.4. FIELD TESTS

3.4.1. General features

Each trial in containers began with an acclimation phase of 24 h before any spraying, to make sure most of the caterpillars would stay on the plants. *Pieris brassicae* starts spreading from stage L2. As caterpillars used were from stage L2 and L3, they were inserted bundled so that they could either keep naturally bundled or start spreading. Caterpillars start eating on the bottom side of leaves. However, to ensure that caterpillars do not fall from the cabbage they were inoculated on the top of the leaf. Leaves were chosen according to their size (i.e. big) and to their location (i.e. above the container). These choices avoided caterpillars to fall easily from the container. For trials with small trays, caterpillars were installed in the tray with cabbage leaves to feed. Holes were performed in trays to allow air to enter. Gauze was installed on the top to avoid caterpillars to leave. Caterpillars were fed with cabbage leaves when needed (i.e. on average three times a week).
About products application, *Bt* biopesticide (L-SSF and commercial solution) needs to be put on the

leaves that caterpillars eat in order to be effective. Thus, biopesticides were applied to the plants with a spray. For the trials on containers, application consisted of carefully spraying all the leaves on both the top and the bottom sides. For the trials with trays, application consisted of carefully spraying the leaves on both sides as well before adding them to feed the caterpillars. L-SSF was used as received from UAB, without any dilution. Commercial *Bt* was used according to manufacturer's instructions. Both products were only sprayed once, just 24 h after caterpillars' acclimation. A control was always used with no product sprayed.
At the end of each cabbage trial all cabbages were removed and next trial was installed with new plants and containers.
Pieris brassicae were counted every day from the day the product was sprayed and during 10 days, except weekends. After 10 days, data were collected every 2 days until the end of the trial.
As caterpillars could leave the cabbage during the experiment, the first data collected were the numbers of *Pieris brassicae* observed. Then, numbers of *Pieris* were separated between alive and dead, as shown in Figure 33. Dead *Pieris* are characterized by no movement and hunched. Alive *Pieris* are moving by themselves or when they are touched.

i: Alive ii: dead

Figure 33: Caterpillar health condition characterisation – photos of dead and alive caterpillars.

After collecting the data, *Pieris* dead and chrysalis were taken out of the experimentation.
When *Pieris brassicae* were found out of the experimental field, they were taken out and considered as "disappeared" as it was complicated to determine from which treatment they belonged.

3.4.2. Biopesticide trial

It was a challenge to find a sensitive-to-*Bt* pest supplier that could provide us the quantity needed. Moreover, *Pieris brassicae* can only be sold part of the year as caterpillars. Thus, the trials were run only for a very short time.
Experimental designs were as summarized in
Table 8.

Table 8: List of treatments in Biopesticide field test

Trial	Containers or Trays	Duration of trial	Number of container or tray	Caterpillars per container or tray	Product
Trial 1-Containers-2018-10-09	Containers	13 days	10	10	L-SSF
					Commercial *Bt*
					-
Trial 2 -	Containers		9		L-SSF

Containers-2018-10-17		13 days		7	Commercial *Bt*
					-
Trial 3 –Trays - 2018-10-17	Trays	12 days	15	6	L-SSF
					Commercial *Bt*
					-

For trial in containers, cabbages were grouped as in Figure 34 and Figure 35.

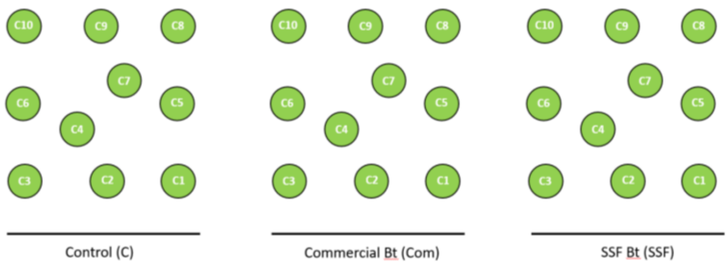


Figure 34: Trial 1 experimental set-up on cabbages

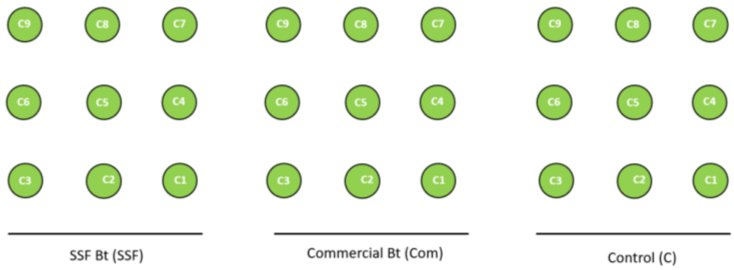


Figure 35: Trial 2 experimental set-up on cabbages

Apart from number of replicates, Trial 1 and 2 were identical. Indeed, it was decided that further data were needed to strengthen the results, especially as we suspected some of the caterpillars' death to be caused by another reason than the pesticide.

3.5. STATISTICAL METHODS

The biopesticide experimentation aimed to compare the caterpillars' mortality rate obtained for each treatment. Therefore, an association test for qualitative variables was used. The objective was to find if the variable product and caterpillars' mortality were independent or dependent. Percentages of mortality were studied. Cabbage and trays were not considered as a replicate because caterpillars of the same treatment tended to move from a cabbage to another. Therefore, caterpillars were considered as the replicate.

As measures were independent, Pearson's Chi-squared test was conducted when all numbers of samples were larger than 5 and the global number was 20. In the case of any number less than 5, a Fisher test was conducted. The hypothesis Ho was: "Product and mortality were independent". The alternative hypothesis H1 was: "Product and mortality were dependent". The limit of acceptation of Ho was 5 %. In addition, the results gave also the difference between treatments and the confidence interval at 95 %, available in Annex 8.

4. Results and discussion

P-values are not indicated in this section, for additional information on p-values, please see Annex 8, where they are all referred.

4.1. TRIAL 1 IN CONTAINERS

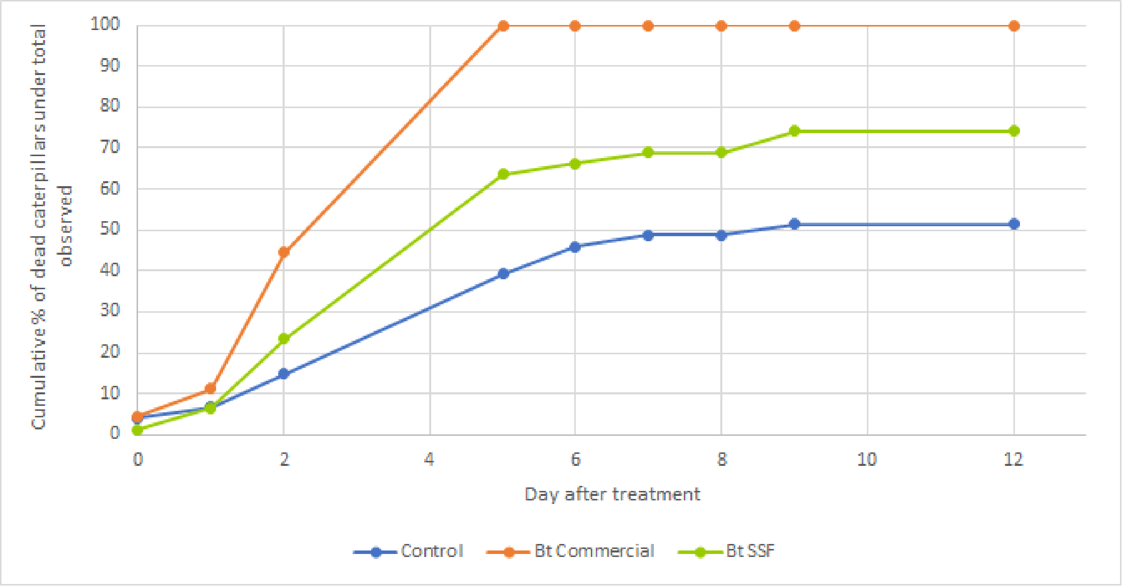
The goal of Trials 1 and 2 was to compare the quantity of dead caterpillars obtained with L-SSF, Commercial *Bt* and Control to understand if the mortality of the caterpillars depended on the product sprayed.

Products were sprayed on day 0 and the trial ran until day 12. The results are presented in Table 9 and Figure 36.

Table 9: Trial 1 – Percentage of dead, alive and disappeared caterpillars

	Control	Commercial *Bt*	L-SSF
Alive	36 %	0 %	20 %
Dead	38 %	90 %	57 %
Disappeared	26 %	10 %	23 %

Table 9 shows a percentage of disappeared caterpillars, corresponding to the difference between the inoculated caterpillars and the sum of alive and dead caterpillars observed at the end of the trial. It was supposed that disappeared caterpillars were either dead or alive, but outside of the cabbage container. The following analysis was done considering only the observed caterpillars. It was supposed that more caterpillars disappeared in Control and L-SSF as they stayed alive longer time.



- L-SSF led to significantly higher mortality rate than Control of +23 %.
- L-SSF led to significantly lower mortality rate than Commercial *Bt* of -26 %.
- Commercial *Bt* led to significantly higher mortality rate than Control of +49 %.

Figure 36: Trial 1 –Cumulated evolution of observed caterpillars' mortality rate

As shown on Figure 36, at the end of the trial, Commercial *Bt* induced 100 % of mortality, when L-SSF reached 74 % and Control 51 %.

For all treatments, the highest mortality rate observed was between 2 and 5 days after product was sprayed with 63 % for Control, 89 % for Commercial *Bt* and 77 % for L-SSF. The high mortality rate of Control is discussed later in the paragraph "Discussions".

In Trial 1, L-SSF led to a higher mortality rate than Control (23 %) and a lower mortality rate than Commercial *Bt* (-26 %).

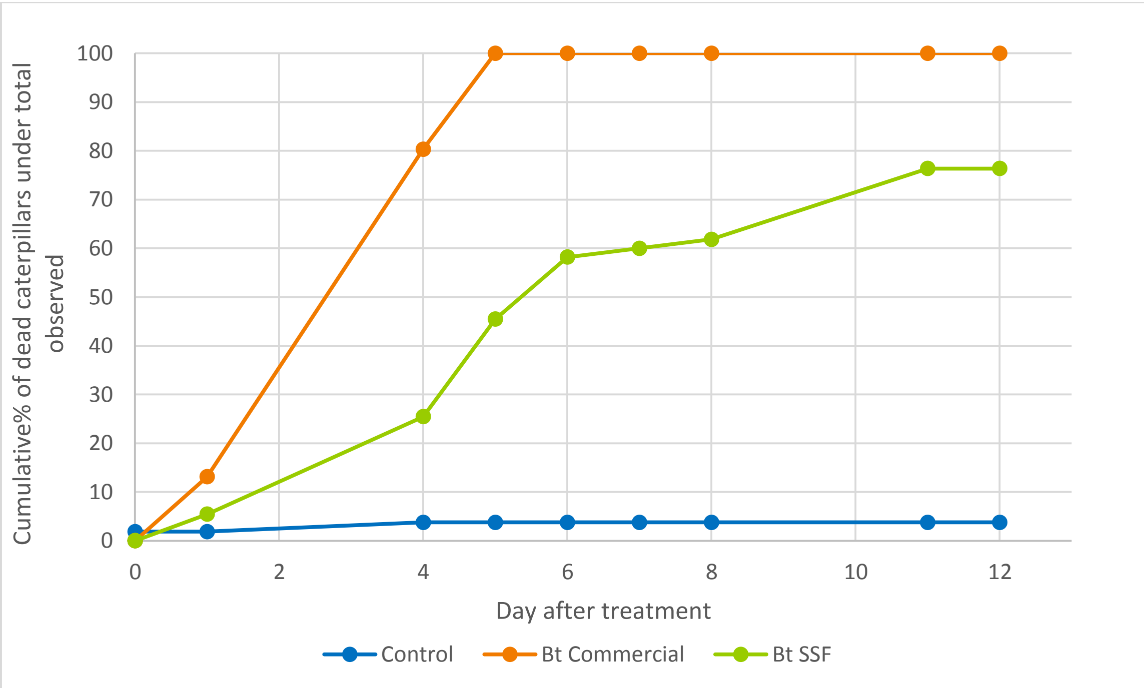
4.2. TRIAL 2 IN CONTAINERS

Products were sprayed on day 0 and the trial ran until day 12. The results are presented in Table 10 and Figure37.

Table 10: Trial 2 - Percentage of dead, alive and disappeared caterpillars

	Control	Commercial *Bt*	L-SSF
Alive	81 %	0 %	17 %
Dead	3 %	97 %	70 %
Disappeared	16 %	3 %	13 %

Table 10 shows a percentage of disappeared caterpillars, corresponding to the difference between the inoculated caterpillars and the sum of alive and dead caterpillars observed at the end of the trial. It was supposed that disappeared caterpillars were either dead or alive, but outside of the cabbage container. The following analysis was done considering only the observed caterpillars.



- L-SSF led to significantly higher mortality rate than the Control of +76 %.
- L-SSF led to significantly lower mortality rate than the Commercial *Bt* of -20 %.
- Commercial *Bt* led to significantly higher mortality rate than the Control of +96 %.

Figure 37: Trial 2 - Cumulated evolution of observed caterpillars' mortality rate

As shown Figure 37, at the end of the trial, Commercial *Bt* induced 100 % of mortality, when L-SSF reached 76 % and Control 4 %.

Commercial *Bt* reached 100 % of dead caterpillars within the 5 days following the product application. L-SSF had the highest mortality rate observed from day 1 to day 6 with 60 % of the dead caterpillars.

In Trial 2, L-SSF led to a higher mortality rate than Control (76 %) and a lower mortality rate than Commercial *Bt* (-20 %). Mortality rate in Control was low compared to Trial 1.

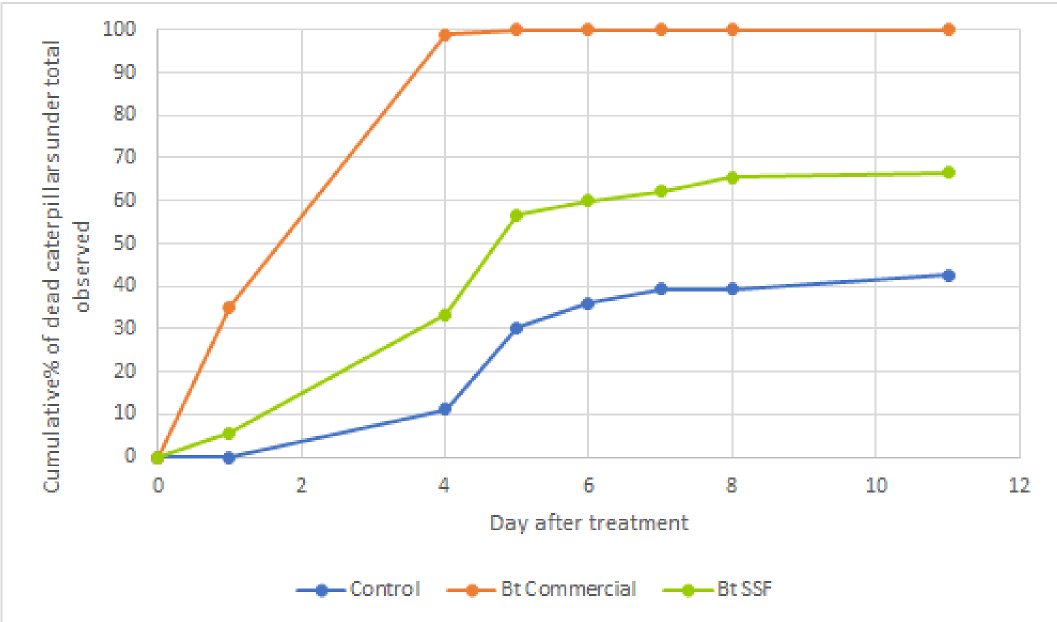
4.3. TRIAL 3 IN TRAYS

Products were sprayed on day 0 and the trial ran until day 11. The results are presented in Table 11 and Figure 38.

Table 11: Trial 3 - Percentage of dead, alive and disappeared caterpillars

	Control	Commercial *Bt*	L-SSF
Alive	57 %	0 %	33 %
Dead	42 %	100 %	67 %

Table 11 shows that no caterpillars disappeared. Indeed, the small trays system prevented from any caterpillars getting eaten or going out of the experimental field.



- L-SSF led to significantly higher mortality rate than the Control of +24 %.
- L-SSF led to significantly lower mortality rate than the Commercial *Bt* of -33 %.
- Commercial *Bt* led to significantly higher mortality rate than the Control of +57 %.

Figure 38: Trial 3 - Cumulated evolution of observed caterpillars' mortality rate

As shown on Figure 38, at the end of the trial, Commercial *Bt* induced 100 % of mortality, when L-SSF reached 67 % and Control 43 %.

Commercial *Bt* reached 100 % mortality rate in 5 days. L-SSF had the highest mortality rate observed from day 1 to day 5 with 85 % of the dead caterpillars.

In Trial 3, L-SSF led to a higher mortality rate than Control (24 %) and a lower mortality rate than Commercial *Bt* (-33 %).

4.4. DISCUSSION

4.4.1. Mortality rate observed on Control

There was a high mortality rate observed on the treatment Control for both Trial 1 and Trial 3 when mortality rate was very low for Trial 2. First, Commercial *Bt* sprayed was suspected to be responsible of the mortality rate observed for the Control. Indeed, during the trials Commercial *Bt* treatment was located between Control and L-SSF. Spray could have contaminated the closer cabbages in the Control and for L-SSF. However, the sprayer was very careful, and the mortality rate of the caterpillars was not higher on

Control cabbages neighbour to Commercial *Bt* cabbages than the other plants of the treatment. Besides, there was very low mortality rate in Control Trial 2 on cabbages, though configuration was the same. Thus, that should not be the origin of the high mortality rate.
During Trial 3 in trays, an incident happened. Cabbages leaves dried in fewer time than expected leaving caterpillars without food in some small trays on Control treatment, which could have caused some deaths. Also, caterpillars in small trays were stored in a room at cold night temperature, which could have increased the mortality rate. In small trays trial was useful because compared to cabbages, caterpillars could not disappear. However, this high mortality rate prevents us to conclude efficiently on this effect.
For Trial 1 in containers the changes of trial planning caused by caterpillar disappearance led to a lack of plants. Cabbages were reused from previous tests that had already been sprayed by Commercial *Bt*, L-SSF or cabbages that were not sprayed at all. However, it was decided to use them again as *Bt* persistence is known to be very low and sprayed were done more than 3 weeks before. Indeed, the toxin is destroyed by UV radiation and product loose efficiency within a few hours. Seeing the mortality rate on Control, spray was suspected to have impacted the deaths. On Trial 2 in containers, plants were younger and had not been used in biopesticide trials before. They were maybe smoother and more palatable to *Pieris brassicae*.
Some deaths could have occurred when disposing the caterpillars. Tweezers were used to manipulate the caterpillars and could have hurt them thus leading them to die few days after acclimation. Another hypothesis is transport conditions. Caterpillars were received by post, which could have weakened them during warm days.
Finally, mortality rates of Control and L-SSF display parallel trends even though L-SSF mortality was higher. That means they had common mortality effect variable and that the difference could be caused by the biopesticide effect of L-SSF.

4.4.2. Products efficiency

L-SSF resulted in 67 to 76 % of mortality rate, Commercial *Bt* 100 % and Control 4 to 51 %. Thus, Commercial *Bt* was extremely efficient with all observed caterpillar dead. L-SSF had always a mortality rate significantly higher than the Control (23 to 76 %) and lower than Commercial *Bt* (-33 to -20 %).
Dead caterpillars from L-SSF treatment have similar symptoms as dead caterpillar from Commercial *Bt*, which means that L-SSF and Commercial *Bt* had the same mechanism of action. Thus, *Bt* contained in L-SSF seemed to affect caterpillars. However, it was not as efficient as Commercial *Bt*. It could be because in Commercial *Bt*, the toxin acts directly on the pest whereas in L-SSF, the bacteria needs to form the toxin first. Besides, a hypothesis is that toxins partly stay inside the cell and are not completely released to the media. A cell lysis would be necessary in order to release all toxins to the media.
Commercial *Bt* concentration is indicated in UIAK/g, while to quantify the activity of the substance in the L-SSF Bt *concentration* is measured in CFU/ml. which is the number of bacteria colonies. Those two measurements cannot be easily compared. Amounts of toxin sprayed could have been lower in L-SSF compared to Commercial *Bt*.
Another hypothesis is that L-SSF does not contain adjuvant and could therefore evaporate or be cleaned more easily by irrigation than Commercial *Bt*.
Besides, previous studies showed that entomotoxicity of *Bt* depends on the concentration of heavy metals and especially of Cu (II) (Zhuang *et al.*, 2010). The spray used to spray *Bt* had also been used to spray Cu (II) in the experimentation of solid fertilizer in wooden container to fight mildew. Even if it has been strongly cleaned, the spray might have kept traces of cupper that could have decrease L-SSF efficiency. Moreover, traces of Cu (II) might come from the impurities in the biowaste used to obtain digestate and then L-SSF.

4.4.3. Commercial *Bt* acts faster than L-SSF

L-SSF was mostly efficient during 5 to 6 days after it was sprayed, with then fewer dead caterpillars. Commercial *Bt* was 100 % efficient 5 days after product was sprayed the latest. In Trial 3 in trays, there were already 32 and 58 dead the two first days after spraying Commercial *Bt* when it was only 5 and 25 dead for L-SSF. However, it is not possible to determine which day was the most efficient for both products as days 3 and 4 were during weekend and caterpillars were not counted on these days.
Once again, the reason can be that in Commercial *Bt*, the toxin is in crystal form and acts directly. In L-SSF the toxicity of the bacillus comes after the liberation of the crystal and its transformation into toxin. That could explain why L-SSF toxin takes longer time to act compared to Commercial *Bt* toxin.
Moreover, caterpillars keep growing during this difference of time and can become more resilient to the toxin.

4.4.4. Disappeared caterpillars

Even if nets have been used in containers trials, caterpillars still disappeared out of the experimentation field. A first hypothesis was that some pests consumed the caterpillar. A second hypothesis was that caterpillars were going by themselves out of the experimentation field. Indeed, few caterpillars were found in the greenhouse outside the net or walking on the top edge of the nets. Caterpillars could leave from stage L4 because of a lack of food on their cabbage or a lack of space. Indeed, caterpillars started spreading from stage L2-L3. This is particularly true for Trial 2 in containers, where cabbages were smaller than in Trial 1. Indeed, the caterpillars on Control treatments had no more cabbage to eat after 10 days and were moving from a cabbage to another. The last hypothesis could be that dead caterpillars were not found in the container due to their colour, similar to the soil. Irrigation could have moved the upper part of the soil and have recovered and so hidden dead caterpillars.

5. Conclusion and outlook

5.1. CONCLUSION

An overview of the Biopesticide field test results is presented in Figure 30 and Figure 31.

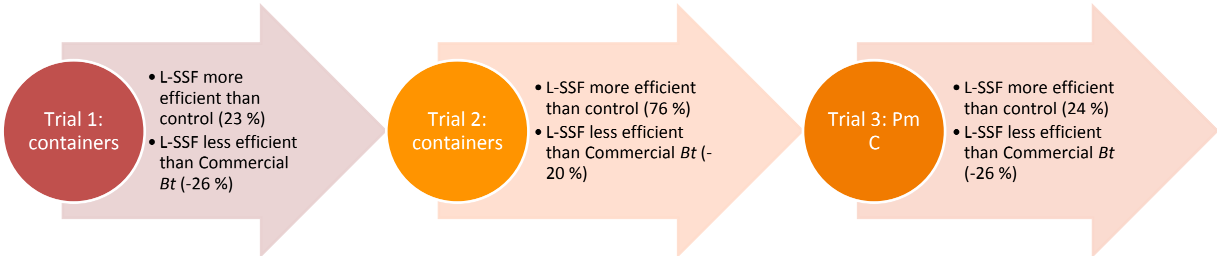


Figure 39 : Overview of Biopesticide field test results

L-SSF was proved to be efficient as a biopesticide with up to 76 % mortality rate. It was always more efficient than Control with no biopesticide in the three trials. However, it was always less efficient (and slower for being effective) than the commercial solution. The commercial solution was extremely efficient, always bringing 100 % death.

There were challenges in caterpillar disappearance and in death caused by other variables than biopesticides. Protocol needs to be improved to have more useable results. Eventually, L-SSF product itself could be improved to increase in efficiency, especially by ensuring that all toxins are released to the media. Further trials will be performed in Season 2019.

1.1. PROTOCOL IMPROVEMENT

Several improvements will be undertaken:

- New sprays will be used, and sprays will be specific for each product to avoid contamination by copper or another product.
- Besides, bigger plants will be ordered to have enough food for caterpillars during the whole experiment for them not to move away or to another treatment.
- Plants will not be reused from a trial to another.
- Caterpillars will be installed and sprayed on Mondays in order to count them the first five days.
- Irrigation will change from watering the plant to sub irrigation: water will not be sprayed on the surface but will moisten the container from the bottom, avoiding hiding dead caterpillar body on the soil.
- Location of the three products will be spread on three different big trays. Even though it was excluded that Commercial *Bt* would be the reason of high mortality rate in Control, it is better to be on the safe side.
- Trials on small trays will be performed again. There will be a focus on checking that enough fresh cabbage leaves are available to feed caterpillars and that the temperature keeps warm at night.

1.2. FURTHER TRIALS ON L-SSF EFFICIENCY

Commercial *Bt* is advised to be reused after 7 days if not completely efficient. This did not happen as all caterpillars died after the first spray. However, this protocol will be applied on L-SSF. Indeed, its efficiency seems to decrease after 5 to 6 days and a higher frequency might kill more caterpillars. Products will be sprayed two times, on day 1 and day 4. Increase the frequency of product spraying could allow to reach 100 % of dead caterpillars.

To define the period of action of Commercial *Bt* and compare it with *Bt*, caterpillars will be re-acclimated two days after product is sprayed and check if the product still affects them.

In this first season of trial, L-SSF extracted from S-SSF has been used. S-SSF will be possibly tested as well. Indeed, one of our contacts has regularly challenges with another *lepidoptera* pest: *Duponchellia fovealis*. Its caterpillars live in the substrate or at the plants collars and could be impacted by S-SSF used as a substrate. However, this butterfly is an ornamental plants pest and not a farming one. It still needs to be decided if this study will be in DECISIVE scope or not.

An additional goal is to use material from SSF demonstration plant operation. First SSF reactor will be in Barcelona demonstration site: biopesticide will certainly be imported from there. Then SSF reactor will be on Lyon demonstration site and bioproducts will be directly usable onsite.

1.3. PRODUCT IMPROVEMENT

The commercial products specified the number of active substances, which is not convertible in number of CFU. Thus, the difference of mortality could be caused by a different of toxin amount, which is hard to assess with these differences of units. Working with UAB, further products references will be analysed to determine the quantity of *Bt* necessary to reach 100 % of dead caterpillars in terms of CFU.

Product homogeneity needs to be assessed. In partnership with UAB, chemical and biological analyses will be performed at each product trial before and after shipment to study product homogeneity. It will allow to see transport impact and product homogeneity between batches. Moreover, trials between demonstration sites and lab experiments from UAB will compare their relative efficiency.

Moreover, in partnership with UAB, a cell lysis step could be implemented in the process of product development or product use in order to release all toxins from *Bt* to the media.

Also, a biopesticide more concentrated in *Bt* should be studied. The concentration could be increased with a colder transport, improved conservation and more concentrated extraction. Discussions will be conducted with UAB to explore those possibilities. Moreover, if UAB could provide several concentrations, their efficiency will be compared.

Besides, differences of efficiency according to the type of extraction (i.e. with water or Ringer solution) could be assessed.

General conclusion

This report studied the potential of bioproducts from AD and SSF as inputs in urban agriculture. Biofertilizers and biopesticides coming from DECISIVE technologies, AD and SSF, were tested. This report presented Season 2018 results of three field tests on liquid fertilizers, solid fertilizers and biopesticides.

Liquid fertilizers field test studied on one hand the potential of L-digestate and L-SSF as fertilizers in hydroponics and, on another hand, what were the best mineral and organic commercial fertilizers in order to compare their yields in Season 2019. Results showed that the fertilizer Pm C at EC 1.7 mS/cm was the best mineral fertilizer. The best organic fertilizer was Po B at EC 1.2 mS/cm with 0.25 ml/l of molasses and F1 as a fungal product. Considering L-digestate, the best treatment was EC 1. 2 mS/cm, with 0.35ml/l of molasses, and containing F2 as a fungal product. However, these conclusions need to be validated by means of the currently running Trial 4. Besides, L-SSF could not be assessed properly because of a lack of the product during the trial. In Season 2019 L-digestate will be compared to the best commercial mineral and organic fertilizer. Besides, L-SSF properties will be studied.

Solid fertilizer field test focused on the potential of S-digestate as fertilizer in wooden containers. The experimentation “Substrate” gave the best results. It contained 50 % of the studied products (digestate or compost) mixed with 50 % structuring components. Digestate gave very promising results as fertilizer on the growth of basil and tomato plants, with tomatoes yields as good as the soil control.

In Season 2019 the field test will be run longer with more replicates. Besides, L-SSF could be studied according to the quantities available. Digestate origin impact will be considered as well.

Biopesticide field test assessed the pesticide properties of L-SSF. It led to promising results with L-SSF being more efficient than the control but less efficient than a commercial solution. Challenges of caterpillars disappearing or dying for other reasons appeared.

Season 2019 will focus on both getting rid of those challenges related to caterpillars and improving L-SSF product and its best way of use. Especially the frequency of the spraying will be studied. If relevant, S-SSF biopesticide potential on soil pests will be tested.

Eventually, more laboratory and sensory analyses will be performed in Season 2019 on the three field tests. Chemical composition, heavy metals concentration and sensory analyses will be conducted.

Moreover, the circularity concept of DECISIVE will be assessed in Season 2019-2020 as most of the bioproducts from AD and SSF tested should come from Lyon pilot plant or if needed Barcelona pilot plant. That means herbs will be sold to restaurants whose biowaste will be collected. Biowaste will be treated through AD and SSF process just a few meters from the farm in Lyon and bioproducts made this way will be used to grow and protect herbs, which will be sold to the restaurants.

Bibliography

1. ADEME, 2015. Le compostage. Fiche technique. [**online**] Available on :<https://www.ademe.fr/sites/default/files/assets/documents/fiche-technique-le-compostage-201511.pdf>

2. ADEME, 2018/08/23. La méthanisation [**online**]. Available on: < www.ademe.fr/expertises/dechets/passer-a-laction/valorisation-organique/methanisation> (Consulted the 2018/11/07)

3. ADEME, October 2011. Qualité agronomique et sanitaire des digestats [**online**] Available on : < www.ademe.fr/sites/default/files/assets/documents/79519_qualite_digestat_rf_octobre_2011.pdf>

4. AGRIBIOVAR and Chambre de l'Agriculture du Var. Le point sur le compost, note technique. [online] Available on : < https://paca.chambres-agriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Provence-Alpes-Cote_d_Azur/Fiche_compost.pdf>

5. APREL, Chambre d'agriculture Vaucluse, GRAB, 2017. Liste des produits phytosanitaires autorisés en agriculture biologique sur cultures maraîchères et fraise.

6. Article L255-1 du code rural et de la pêche maritime (France), 2018/11/02. [**online**] Available on :< https://www.legifrance.gouv.fr/affichCode.do;jsessionid=4ECDCCCD7C71B2987CDBD88FF9816162.tplgfr21s_2?idSectionTA=LEGISCTA000030678486&cidTexte=LEGITEXT000006071367&dateTexte=20181108>

7. Article L253-6 du code rural et de la pêche maritime (France), 2018/11/02. [**online**] Available on : < https://www.legifrance.gouv.fr/affichCodeArticle.do?cidTexte=LEGITEXT000006071367&idArticle=LEGIARTI000006583210&dateTexte=&categorieLien=cid>

8. Ballardo, C., Abraham, J., Barrena, R., Artola, A., Gea, T., Sanchez, A., 2015. Valorization of soy wastes though SSF for the production of compost enriched with *Bacillus thuringiensis* with biopesticide properties. Journal of Environmental Management, Volume 169, 15 March 2016, 126-131.

9. Benítez, T., Rincón, A.M., Limón, M.C., Codón, A.C., 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology (2004) 7: 249-260.

10. BIO by Deloitte and TITTMO Agroenvironnement, 2014. Produits de stimulation en agriculture visant à améliorer les fonctionnalités biologiques des sols et des plantes – Etude des connaissances disponibles et recommandations stratégiques.

11. Brouwers, M. and Farinet, J. Utilisation Agricole de sous-produits de la mélasse et fertilisation de la canne à sucre. Composition chimique des sols, Agriculture et développement n°24 60-62.

12. Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P., Piccolo, A., 2015. Humic and fluvic acid as biostimulants in horticulture. Scientia Horticulturae 196 (2015) 15–27.

13. Caron, J., 2002. Le pouvoir antagoniste de *Trichoderma*. Conférence présentée lors des journée horticoles régionales à St-Rémy, le 5 décembre 2002.

14. Cerda, A., El-Bakry, M., Gea, T., Sánchez, A., 2016. Long term enhanced solid-state fermentation: Inoculation strategies for amylase production from soy and bread wastes by *Thermomyces* sp. In a sequential batch operation. Journal of Environmental Chemical Engineering, Vol. 4 Issue 2: 2394-2401.

15. Cerda, A., Mejias, L., Rodríguez, P., Rodríguez, A., Artola, A., Font, X., Gea, T., Sánchez, A., 2018. Valorisation of digestate from biowaste through solid-state fermentation to obtain value added bioproducts: A first approach. Bioresource Technology Volume 271 (2019) 409-416.

16. Castilho, L.R., Mitchell, D.A., Freire, D.M.G., 2009. Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. Bioresource Technology, Vol. 100, Issue 23: 5996-6009.

17. Commission Européenne, 2016. Paquet sur l'économie circulaire. Proposition de règlement du parlement européen et du conseil établissant les règles relatives à la mise à disposition sur le marché des fertilisants porteurs du marquage CE et modifiant les règlements (CE) n°1069/2009 et (CE) n°1107/2009.

18. Commission Regulation (EC) n°1881/2006 setting maximum levels for certain contaminants in foodstuffs **[online]**. Available on : <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:FR:PDF>

19. Commission Regulation (EC) n°889/2008 laying down detail rules for the implementation of Council Regulation (EC) No 834/2207 on organic production and labelling of organic products with regard to organic production, labelling and control. [**online**] Available on: < https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2008.250.01.0001.01.ENG>

20. Daniel, A.C., 2013. Aperçu de l'agriculture urbaine en Europe et en Amérique du Nord.

21. Directive 2009/128/EC of the European Parliament and the Council of 21 October 2009 establishing a framework for the Community action to achieve the sustainable use of pesticides. [**online**] Available on: < https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:EN:PDF>

22. Ducellier G., Isman M., 1955. Dix-huit années de travaux sur le gaz de fumier, Rapport adressé à l'Académie d'agriculture de France, Paris. Biométhane vol. 2 principes, techniques et utilisations, Bernard Lagrange, 1979 Edisud.

23. EBIC, 2018 Available on: < www.biostimulants.fr/produits-utilisation/definition/distinction-biocontrole/ > (Consulted 2018/11/08).

24. El-Bakry, M., Abraham, J., Cerda, A., Barrena, R., Ponsá, S., Sánchez, A., 2015. From Wastes to High Value-Added Products: Novel Aspects of SSF in the Production of Enzymes. Critical Reviews in Environmental Science and Technology. 45(18), 1998-2042.

25. EurObserv'ER, November 2014. Biogas barometer [**online**]. Available on: < www.eurobserv-er.org/biogas-barometer-2014/> (Consulted the 2018/11/08).

26. European Biogas Association, 2017. EBA Statistical Report 2017 [**online**]. Available on: < european-biogas.eu/2017/12/14/eba-statistical-report-2017-published-soon/>

27. European Commission [DG ENV – Directorate C], 2010. Preparatory Study On Food Waste Across EU 27.

28. European Commission, 2019. Fertilizers Working Group (E01320), Register of commission expert groups [online] Available on: http://ec.europa.eu/transparency/regexpert/index.cfm?do=groupDetail.groupDetail&groupID=1320 (Consulted the 2019/02/05).

29. FAO, 2011. Global food losses and food waste – Extent, causes and prevention. Rome. [**online**] Available on: <http://www.fao.org/docrep/014/mb060e/mb060e00.pdf > (consulted on the 15/11/2018).

30. FAO, 2018. Urban agriculture [**online**] Available on :< www.fao.org/urban-agriculture/fr >

(Consulted the 2018/11/08)

31. Fouda, S. 2013, Nitrogen availability of various biogas residues applied to ryegrass. Journal of Plant Nutrition and Soil Science, Vol 176 (4).

32. Furukawa, Y. and Hasegawa, H., 2006. Response of Spinach and Komatsuna to Biogas Effluent Made from Source-Separated Kitchen Garbage. Journal of Environment Quality. Vol 35 (2006), 1939-1947.

33. Girardet, H., 2000. Cities, People, Planet. Liverpool (UK) Schumacher Lectures, urban Sustainability.

34. Grard, B.J.P., Bel, N., Marchal, N., Madre, F., Castell, J.F., Cambrier, P., Houot, S., Manouchehri, N., Besancon, S., Michel, J.C., Chenu, C., Frascaria-Lacoste, N., Aubry, C., 2015. Future of Food: Journal on Food, Agriculture and Society 3 (1) Summer 2015 21- 34.

35. Hingham, E.R., 2005. The Compost Tea Brewing Manual, Fifth Edition. Soil FoodWeb Incorporated.

36. Höfte, H. and Whiteley, H.R., 1989. Isecticidal Crystal Proteins of *Bacillus thuringiensis*. Microbiological Reviews, June 1989, p. 242-255.

37. IBMA, 2017. Le biocontrôle, Comité inter-régional FranceAgriMer Bretagne & Pays de Loire. Vendredi 19 janvier 208. [**online**]. Available on: <http://www.franceagrimer.fr/content/download/55372/535076/file/04%20180119_IBMA_Comit%C3%A9_inter_r%C3%A9gional_FranceAgriMer%20RR%20r%C3%A9gionales%20Bretagne%20Pays%20de%20la%20Loire%20du%2019%20janvier%202018.pdf >

38. Jiménez-Peñalver, P., Gea, T., Sánchez, A., Font, X., 2016. Production of sophrolipids from winterization oil cake by solid-state fermentation: Optimization, monitoring and effect of mixing. Biochemical Engineering Journal. 115, 93-100.

39. L. Avio, C. Sbrana, M. Giovannetti, S. Frassinetti, 2017. Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties. Scientia Horticulturae 224 (2017) p. 265–271

40. Larousse, 2018. Hydroponique [**online**] Available on :< www.larousse.fr> (Consulted the 2018/11/08)

41. Lee and Bartlett, 1976. Stimulation of Plant Growth by Humic Substances.

42. Liedl, B.E., Bombardiere, J., Strowers, A., Mazzaferro, K., Chatfield, J.M., 2015. Liquid effluent from thermophilic anaerobic digestion of poultry litter as a potential fertilizer. HortScience, July 2005. Vol 40 no 4 1132-1133.

43. Liedl, B.E., Cummins, M., Young, A., Williams, M.L., Chatfield, J.M., 2004. Hydroponic lettuce production using liquid effluent from poultry waste bioremediation as nutrient source. ActaHorticulturae 659, 721-728.

44. Liu, W.K., Yang, Q.C. and Du, L., 2009. Soilless cultivation for high-quality vegetable with biogas manure in China: feasibility and benefit analysis. Renewable Agriculture and Food Systems 24(04) 300-307.

45. Loi d'avenir pour l'agriculture l'alimentation et la forêt (LAAF), 2014 October 13th, n°2014-1170, article 50.

46. López-Bucio J., Pelagio-Flores R., Herrera-Estrella A., 2015. Trichoderma as biostimulant: exploring the multilevel properties of a plant beneficial fungus. Scientia Horticulturae 196 109-123.

47. Lošák, T., Zatloukalová, M., Hlušek, J., Fryč J., Vítěz, T., 2010. Comparison of the effectiveness of digestate and mineral fertilizers on yields and quality of kohlrabi (*Brassica oleracea*, L.). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunenis. Vol LIX, No 3, 2011, 117-122.

48. Martin, Y., 2015. Les règles de l'art pour réussir son compost. PleineTerre. [online]. Available on : < https://www.agrireseau.net/documents/Document_91238.pdf>

49. Martínez, O., Sánchez, A., Font, X., Barrena, R., 2017. Valorization of sugarcane bagasse and sugar beet molasses using *Kluyveromyces larxianus* for producing value-added aroma compouds via solid-state fermentation. Journal of Cleaner Production, Vol. 158: 8-17.

50. Ministère de l'agriculture, de l'agroalimentaire et de la forêt (France), 2016. Arrêté du 27 avril 2016 établissant la liste des substances naturelles à usage biostimulant. JO du 30 avril 2016.

51. Ministère de la transition écologique et solidaire (France), mars 2018. Conclusion du groupe de travail « méthanisation » [**online**]. Available on: < www.ecologique-solidaire.gouv.fr/sites/default/files/2018.03.26_DP_Conclusions_methanisation.pdf >

52. Möller, K. and Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth. Eng. Life Science (2012), Vol 12, No. 3, 242-257.

53. Möller, K., Schulz and R., Müller, T., 2010. Substrate inputs, nutrients flows and nitrogen loss of two centralize biogas plants in southern Germany. Nutrient Cycling in Agroecosystems 87 (2), 307-325.

54. Mougeot, L.J.A., 2000. Urban Agriculture: Definition, Presence, Potentials and Risks, and Policy Challenges. Cities Feeding People Series, Report 31, International Devlopment Research Center (IDRC).

55. Nzanza, B., Marais, D., Soudry, P., 2012. Yield and nutrient content of tomato (*Solanum lycopersicum* L.) as influenced by *Trichoderma harzianum* and Glomus mosseae inoculation. Scientia Horticulturae 144 (2012) 55-59.

56. OFEV, EFEN, EFAG, AWEL, 2007. Compost et digestat en Suisse. [**online**] Available on : < www.bafu.admin.ch/bafu/fr/home/themes/dechets/publications-etudes/publications/compost-et-digestat-suisse.html>

57. On, A., Wong, F., Ko, Q., Tweddell, R.J., Antoun, H., Avis, T.J., 2014. Antifungal effects of compost tea microorganisms on tomato pathogens. Biological Control 80 (2015) 63-69.

58. Pane, C., Maria Palese, A., Spaccini, R., Piccolo, A., Celano, G., Zccardelli, M., 2015. Enchancing sustainability of a processing tomato cultivation system by using bioactive compost teas. Scientia Horticulturae 202 (2016) 117-124.

59. Pane, C., Villecco, D and Zaccardelli, M., 2013. Short-Time Response of Microbiological to Wase Compost Amendment of an Intensive Soil in Southern Italy. Communications in Soil Science and Plant Analysis, 44, 2344-2352.

60. Patrick du Jardin, 2012. The Science of Plant Biostimulants – A bibliographic analysis.

61. Pertusatti and Prado, 2007. Buffer capacity of humic acid: Thermodynamic approach.

62. Porcel, A., Aroca R., Cano C., Bago A., Ruiz-Lozano J.M., 2007. *A gene from the arbuscular mycorrhizal fungus Glomus intraradices encoding a binding protein is up-regulated by drought stress in some mycorrhizal plants*. Environmental and Experimental Botany 60 (2007) p. 251–256

63. Regulation (EC) No 2003/2003 relating to fertilizers. Safe and effective fertilizers on the EU market [**online**]. Available on: < https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM%3Al21278>.

64. Rouphalel Y., Franken P., Schneider C., Schwarz D., Giovannetti M., Agnolucci M., De Pascal S., Bonini P., Colla G., 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia Horticulturae 196 (2015) p.91–108

65. Sacchi, V.F., Parenti, P., Hanozet, G.M., Giordana, B., Lüthy, P., Wolfersberger, M.G., 1986. *Bacillus thuringiensis* toxin inhibits K+-gradient-dependent amino acid transport across the brush border membrane of *Pieris brassicae* midgut cells. FEBS LETTERS, Vol 204, number 2, 213 – 218.

66. Sahin, U., Anapali, O., Ercisli, S., 2002. Physico-Chemical and Physical Propertiels of somme Substrates Used in Horticulture. Gartenbauwissenschaft, 67 (2). S55-60

67. Scheuerell and Mahaffee, 2002. Compost Tea: Principles and Prospects For Plant Disease Control. Compost Science & Utilization (2002) Vol. 10, No 4, 313-338.

68. Smith, S.E. and Read, D.J., 2008. Mycorrhizal Symbiosis, Third Edition. Academic Press, London.

69. Specht, K., Siebert, R., Hartmann, I., Freisinger, U.B., Sawicka, M., Werner, A., Thomaier, S., Henckel, D., Walk, H., Dierich, A., 2014. Urban agriculture of the future: an overview of sustainability aspects of food production in and on buildings. Agric Hum Values (2014) 31: 33-51.

70. Terralba, 2018. Pack thés compost O_2 [online] Available on : < http://terralba.fr/the-de-compost-outils-plantes-complements>, consulted the 26/11/2018

71. Thomaier, S., Specht, K., Henckel, D., Dierich, A., Siebert, R., Freisinger, U.B., Sawicka, M., 2014. Farming in and on urban building: Present practice and specific novelties of Zero-Acreage Farming (ZFarming). Renewable Agriculture and Food Systems: 30(1), 43-54.

72. Tomas, L., Larroche, C., Pandey, A., 2013. Current Development in Solid-State Fermentation. Biochemical Engineering Journal 81: 146-161.

73. Tyson, R.V., Simonne, E.H., White, J.M., Lamb, E.M., 2004. Reconciling water quality parameters impacting nitrification in aquaponics: the pH levels. Proc. Fla. State Hort. Soc. 117: 79 – 83

74. Upstart University, 2016. 2040: Hydroponic Nutrients & Fertilizers. [**online**] Available on: https://university.upstartfarmers.com/courses/2040-hydroponic-nutrients-fertilizers

75. Vea, E.B., Romeo, D., Thomsen, M., 2018. Biowaste valorization in a future circular bioeconomy. 25th CIRP Life Cycle Engineering (LCE) Conference, 30 April – 2 May 2018, Copenhagen, Denmark. [**online**]. Available on : < https://pure.au.dk/ws/files/127949419/Vea_et_al_2018.pdf>

76. Veenhuizen, R.V., 2006. Cities farming for the future: Urban agriculture for green and productive cities. ETC – Urban Agriculture.

77. Véron, 2007. La moitié de la population mondiale vit en ville. Population & Société. N°435 [**online**]. Available on : <https://www.ined.fr/fichier/s_rubrique/19103/435.fr.pdf>

78. Vishal J. P., Aniruddha, B., 2013. Solid State Fermentation (SSF) for the Production of Sophrolipids from *Starmerella bombicola* NRRL Y-17069 using glucose, wheat bran and oleic acid. Current Trends in Biotechnology and Pharmacy, Vol. 6, Issues 4: 418-424.

79. Wortman, S.E. Crop physiological response to nutrient solution electrical conductivity and pH in an ebb-and-flow hydroponic system. Scientia Horticulturae 194: 34-42.

80. WRAP, Quested., T., Johnson, H., 2009. Household Food and Drink Waste in UK. Report prepared by WRAP. Bandury.

81. Yu & al, 2009. Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta

Agricultura Scandinavica, Section B -Soil & Plant Science, Vol 60 (2010) 262-268.

82. Zaidi, N.W., Singh, M., Kumar, S., Sangle, U.R., Nityanand, Singh, R., Sachitanand, Prasad, R., Singh, S.S., Singh, S., Yadav, A.K., Singh, A., Waza, S.A., Singh, U.S., 2017. Trichoderma harzianum improves the performance of stress-tolerant rice varieties in rainfed ecologies of Bihar, India. Field Crops Research 220 (2018) 97-104.

83. Zhang, F., Meng, X., Yang, X., Ran, W., Shen, Q., 2014. Quantification and role of organic acids in cucumber toot exudates in *Trichoderma harzianum* T-E5 colonization. Plant Physiology and Biochemistry 83 (2014) 250-257.

84. Zhang, W., Qui, L., Gong, A., Cao, Y., Wand, B., 2013. Solid-state Fermentation of Kitchen Waste for Production of *Bacillus thuringiensis*-based Bio-pesticide. BioRessources 8(1), 1124-1135.

85. Zhuang, LI., Zhou, S., Wang, Y., Liu, Z., Xu, R., 2010. Cost-effective production of *Bacillus thuringiensis* biopesticides by solid-state fermentation using wastewater sludge: Effect of heavy metals. Bioresource Technology 102 (2011) 4820-4826.

Annexes

ANNEX 1: P-VALUES FOR STATISTICAL TESTS FOR LIQUID FERTILIZERS REPORT

- **Mineral fertilizations: p-values**

HP – Trial 2018-04-26- Mineral fertilizers-Welch ANOVA & Games Howell test- MASS

	p
PminA_EC2-PminA_EC1	.002
PminB_EC1-PminA_EC1	.004
PminB_EC2-PminA_EC1	<.001
PminC_EC1-PminA_EC1	1
PminC_EC2-PminA_EC1	.731
PminB_EC1-PminA_EC2	1
PminB_EC2-PminA_EC2	.042
PminC_EC1-PminA_EC2	<.001
PminC_EC2-PminA_EC2	.246
PminB_EC2-PminB_EC1	.053
PminC_EC1-PminB_EC1	.001
PminC_EC2-PminB_EC1	.312
PminC_EC1-PminB_EC2	<.001
PminC_EC2-PminB_EC2	.002
PminC_EC2-PminC_EC1	.627

HP – Trial 2018-04-26- Mineral fertilizers-Welch ANOVA & Games Howell test- DIAMETER

	p
PminA_EC2-PminA_EC1	<.001
PminB_EC1-PminA_EC1	.001
PminB_EC2-PminA_EC1	<.001
PminC_EC1-PminA_EC1	1
PminC_EC2-PminA_EC1	.878
PminB_EC1-PminA_EC2	.971
PminB_EC2-PminA_EC2	.776
PminC_EC1-PminA_EC2	<.001
PminC_EC2-PminA_EC2	.021
PminB_EC2-PminB_EC1	.496
PminC_EC1-PminB_EC1	<.001
PminC_EC2-PminB_EC1	.087
PminC_EC1-PminB_EC2	<.001
PminC_EC2-PminB_EC2	.007
PminC_EC2-PminC_EC1	.752

HP- Trial 2018-06-21-Mineral fertilizers- Kurskal-Wallis and Wilcoxon test-MASS

	PminB_EC1	PminB_EC1CT	PminB_EC2	PminB_EC2CT	PminB_EC3	PminB_EC3CT	PminC_EC1	PminC_EC1CT	PminC_EC2	PminC_EC2CT	PminC_EC3
PminB_EC1CT	4.3e-06	-	-	-	-	-	-	-	-	-	-
PminB_EC2	1.4e-07	1.1e-08	-	-	-	-	-	-	-	-	-
PminB_EC2CT	2.7e-05	2.6e-08	1.0000	-	-	-	-	-	-	-	-
PminB_EC3	8.6e-06	4.5e-08	0.9116	0.1173	-	-	-	-	-	-	-
PminB_EC3CT	7.5e-05	4.5e-09	1.0000	1.0000	0.4914	-	-	-	-	-	-
PminC_EC1	9.1e-06	1.0000	2.4e-08	2.5e-08	7.3e-08	6.4e-09	-	-	-	-	-
PminC_EC1CT	2.1e-06	1.0000	9.9e-09	2.5e-08	3.1e-08	2.8e-09	0.1348	-	-	-	-
PminC_EC2	3.6e-08	2.1e-09	1.0000	1.0000	1.0000	1.0000	2.4e-09	1.9e-09	-	-	-
PminC_EC2CT	0.8378	0.0018	2.1e-07	4.7e-06	1.0e-06	3.8e-06	0.0207	7.4e-05	2.5e-08	-	-
PminC_EC3	1.5e-05	2.1e-08	0.0087	0.0059	1.0000	0.0098	6.0e-08	1.2e-08	0.4018	2.0e-06	-
PminC_EC3CT	1.2e-06	2.1e-09	1.0000	0.6255	1.0000	1.0000	2.4e-09	1.9e-09	1.0000	1.0e-07	1.0000

HP- Trial 2018-06-21-Mineral fertilizers- Kurskal-Wallis and Wilcoxon test-DIAMETER

	PminB_EC1	PminB_EC1CT	PminB_EC2	PminB_EC2CT	PminB_EC3	PminB_EC3CT	PminC_EC1	PminC_EC1CT	PminC_EC2	PminC_EC2CT	PminC_EC3
PminB_EC1CT	0.00099	-	-	-	-	-	-	-	-	-	-
PminB_EC2	1.1e-08	1.0e-08	-	-	-	-	-	-	-	-	-
PminB_EC2CT	2.1e-07	1.7e-07	1.00000	-	-	-	-	-	-	-	-
PminB_EC3	2.6e-05	2.0e-06	1.00000	1.00000	-	-	-	-	-	-	-
PminB_EC3CT	0.00011	2.1e-06	1.00000	1.00000	0.06702	-	-	-	-	-	-
PminC_EC1	2.3e-05	1.00000	1.8e-09	2.4e-08	7.9e-07	4.3e-07	-	-	-	-	-
PminC_EC1CT	6.2e-06	1.00000	1.8e-09	2.4e-08	2.4e-07	8.4e-08	1.00000	-	-	-	-
PminC_EC2	3.1e-08	1.1e-08	1.00000	1.00000	1.00000	0.22852	2.8e-09	1.9e-09	-	-	-
PminC_EC2CT	1.00000	0.15315	4.8e-08	6.0e-07	1.1e-05	0.00014	0.02896	0.00123	4.6e-08	-	-
PminC_EC3	2.6e-06	3.0e-07	0.01423	0.00497	0.12791	0.00022	1.9e-07	8.5e-08	0.08868	2.1e-06	-
PminC_EC3CT	2.1e-08	4.3e-09	0.00157	0.00055	0.04194	1.1e-05	2.5e-09	1.9e-09	0.02026	1.3e-08	1.00000

HP – Trial 2018-06-21 – Pm B & Pm C – Kruskal-Wallis and Wilcoxon test – MASS

	PminB_EC1	PminB_EC1CT	PminB_EC2	PminB_EC2CT	PminB_EC3	PminB_EC3CT	PminC_EC1	PminC_EC1CT	PminC_EC2	PminC_EC2CT	PminC_EC3
PminB_EC1CT	4.3e-06	-	-	-	-	-	-	-	-	-	-
PminB_EC2	1.4e-07	1.1e-08	-	-	-	-	-	-	-	-	-
PminB_EC2CT	2.7e-05	2.6e-08	1.0000	-	-	-	-	-	-	-	-
PminB_EC3	8.6e-06	4.5e-08	0.9116	0.1173	-	-	-	-	-	-	-
PminB_EC3CT	7.5e-05	4.5e-09	1.0000	1.0000	0.4914	-	-	-	-	-	-
PminC_EC1	9.1e-06	1.0000	2.4e-08	2.5e-08	7.3e-08	6.4e-09	-	-	-	-	-
PminC_EC1CT	2.1e-06	1.0000	9.9e-09	2.5e-08	3.1e-08	2.8e-09	0.1348	-	-	-	-
PminC_EC2	3.6e-08	2.1e-09	1.0000	1.0000	1.0000	1.0000	2.4e-09	1.9e-09	-	-	-
PminC_EC2CT	0.8378	0.0018	2.1e-07	4.7e-06	1.0e-06	3.8e-06	0.0207	7.4e-05	2.5e-08	-	-
PminC_EC3	1.5e-05	2.1e-08	0.0087	0.0059	1.0000	0.0098	6.0e-08	1.2e-08	0.4018	2.0e-06	-
PminC_EC3CT	1.2e-06	2.1e-09	1.0000	0.6255	1.0000	1.0000	2.4e-09	1.9e-09	1.0000	1.0e-07	1.0000

HP – Trial 2018-06-21 – Pm B & Pm C – Kruskal-Wallis and Wilcoxon test – DIAMETER

	PminB_EC1	PminB_EC1CT	PminB_EC2	PminB_EC2CT	PminB_EC3	PminB_EC3CT	PminC_EC1	PminC_EC1CT	PminC_EC2	PminC_EC2CT	PminC_EC3
PminB_EC1CT	0.00099	-	-	-	-	-	-	-	-	-	-
PminB_EC2	1.1e-08	1.0e-08	-	-	-	-	-	-	-	-	-
PminB_EC2CT	2.1e-07	1.7e-07	1.00000	-	-	-	-	-	-	-	-
PminB_EC3	2.6e-05	2.0e-06	1.00000	1.00000	-	-	-	-	-	-	-
PminB_EC3CT	0.00011	2.1e-06	1.00000	1.00000	0.06702	-	-	-	-	-	-
PminC_EC1	2.3e-05	1.00000	1.8e-09	2.4e-08	7.9e-07	4.3e-07	-	-	-	-	-
PminC_EC1CT	6.2e-06	1.00000	1.8e-09	2.4e-08	2.4e-07	8.4e-08	1.00000	-	-	-	-
PminC_EC2	3.1e-08	1.1e-08	1.00000	1.00000	1.00000	0.22852	2.8e-09	1.9e-09	-	-	-
PminC_EC2CT	1.00000	0.15315	4.8e-08	6.0e-07	1.1e-05	0.00014	0.02896	0.00123	4.6e-08	-	-
PminC_EC3	2.6e-06	3.0e-07	0.01423	0.00497	0.12791	0.00022	1.9e-07	8.5e-08	0.08868	2.1e-06	-
PminC_EC3CT	2.1e-08	4.3e-09	0.00157	0.00055	0.04194	1.1e-05	2.5e-09	1.9e-09	0.02026	1.3e-08	1.00000

HP – Trial 2018-08-31 – Pm C – ANOVA & Tukey HSD tests – MASS

	p
EC2-EC1	.915
EC3-EC1	.066
EC4-EC1	.378
EC5-EC1	<.001
EC6-EC1	.01
EC3-EC2	.488
EC4-EC2	.936
EC5-EC2	.017
EC6-EC2	.162
EC4-EC3	.961
EC5-EC3	.673
EC6-EC3	.989
EC5-EC4	.192
EC6-EC4	.69
EC6-EC5	.955

HP – Trial 2018-08-31 – Pm C – Kruskal Wallis & Wilcoxon tests – DIAMETER

	EC1	EC2	EC3	EC4	EC5
EC2	1.0000	-	-	-	-
EC3	4.5e-05	0.4319	-	-	-
EC4	0.0012	1.0000	1.0000	-	-
EC5	7.4e-06	0.0274	0.5805	0.0193	-
EC6	3.5e-06	0.2201	1.0000	1.0000	0.7130

- **Organic fertilization: p-values**

HP – Trial 2018-04-26- Po C -Kruskal Wallis test & Wilcoxon test- MASS

	EC1	EC1_F1	EC1_F2	EC2	EC2_F1
EC1_F1	0.01956	-	-	-	-
EC1_F2	0.01877	1.00000	-	-	-
EC2	5.4e-08	0.01774	0.00011	-	-
EC2_F1	9.4e-07	0.17205	0.00413	1.00000	-
EC2_F2	0.00032	1.00000	1.00000	0.00789	0.17268

HP – Trial 2018-04-26- Po C -Kruskal Wallis test & Wilcoxon test- DIAMETER

	EC1	EC1_F1	EC1_F2	EC2	EC2_F1
EC1_F1	0.03593	-	-	-	-
EC1_F2	0.12283	1.00000	-	-	-
EC2	6.2e-07	0.25792	3.8e-05	-	-
EC2_F1	1.8e-05	1.00000	0.00267	1.00000	-
EC2_F2	0.00098	1.00000	0.34674	0.00639	0.32855

HP – Trial 2018-04-26- Po B & Po A – Welch ANOVA & Games Howell test- MASS

	p
PorgA_EC1_F1-PorgA_EC1	.007
PorgA_EC1_F2-PorgA_EC1	.412
PorgA_EC2-PorgA_EC1	.002
PorgA_EC2_F1-PorgA_EC1	<.001
PorgA_EC2_F2-PorgA_EC1	<.001
PorgB_EC1-PorgA_EC1	<.001
PorgB_EC1_F1-PorgA_EC1	.001
PorgB_EC1_F2-PorgA_EC1	<.001
PorgB_EC2-PorgA_EC1	.643
PorgB_EC2_F1-PorgA_EC1	<.001
PorgB_EC2_F2-PorgA_EC1	.067
PorgA_EC1_F2-PorgA_EC1_F1	.96
PorgA_EC2-PorgA_EC1_F1	1
PorgA_EC2_F1-PorgA_EC1_F1	.008
PorgA_EC2_F2-PorgA_EC1_F1	.999
PorgB_EC1-PorgA_EC1_F1	.068
PorgB_EC1_F1-PorgA_EC1_F1	1
PorgB_EC1_F2-PorgA_EC1_F1	.44
PorgB_EC2-PorgA_EC1_F1	.992
PorgB_EC2_F1-PorgA_EC1_F1	.033
PorgB_EC2_F2-PorgA_EC1_F1	.989
PorgA_EC2-PorgA_EC1_F2	.747
PorgA_EC2_F1-PorgA_EC1_F2	<.001
PorgA_EC2_F2-PorgA_EC1_F2	.49
PorgB_EC1-PorgA_EC1_F2	.003
PorgB_EC1_F1-PorgA_EC1_F2	.717
PorgB_EC1_F2-PorgA_EC1_F2	.048
PorgB_EC2-PorgA_EC1_F2	1
PorgB_EC2_F1-PorgA_EC1_F2	.001
PorgB_EC2_F2-PorgA_EC1_F2	.692
PorgA_EC2_F1-PorgA_EC2	.025
PorgA_EC2_F2-PorgA_EC2	1
PorgB_EC1-PorgA_EC2	.208
PorgB_EC1_F1-PorgA_EC2	1
PorgB_EC1_F2-PorgA_EC2	.752
PorgB_EC2-PorgA_EC2	.903
PorgB_EC2_F1-PorgA_EC2	.122
PorgB_EC2_F2-PorgA_EC2	1
PorgA_EC2_F2-PorgA_EC2_F1	.044
PorgB_EC1-PorgA_EC2_F1	.993
PorgB_EC1_F1-PorgA_EC2_F1	.022
PorgB_EC1_F2-PorgA_EC2_F1	.823
PorgB_EC2-PorgA_EC2_F1	.001
PorgB_EC2_F1-PorgA_EC2_F1	.996
PorgB_EC2_F2-PorgA_EC2_F1	.633
PorgB_EC1-PorgA_EC2_F2	.325
PorgB_EC1_F1-PorgA_EC2_F2	1
PorgB_EC1_F2-PorgA_EC2_F2	.88
PorgB_EC2-PorgA_EC2_F2	.741
PorgB_EC2_F1-PorgA_EC2_F2	.208
PorgB_EC2_F2-PorgA_EC2_F2	1
PorgB_EC1_F1-PorgB_EC1	.184
PorgB_EC1_F2-PorgB_EC1	1
PorgB_EC2-PorgB_EC1	.011
PorgB_EC2_F1-PorgB_EC1	1
PorgB_EC2_F2-PorgB_EC1	.983
PorgB_EC1_F2-PorgB_EC1_F1	.726
PorgB_EC2-PorgB_EC1_F1	.893
PorgB_EC2_F1-PorgB_EC1_F1	.105
PorgB_EC2_F2-PorgB_EC1_F1	.999
PorgB_EC2-PorgB_EC1_F2	.111
PorgB_EC2_F1-PorgB_EC1_F2	.999
PorgB_EC2_F2-PorgB_EC1_F2	1
PorgB_EC2_F1-PorgB_EC2	.005
PorgB_EC2_F2-PorgB_EC2	.788
PorgB_EC2_F2-PorgB_EC2_F1	.967

HP – Trial 2018-04-26- Po B & Po A – Wilcoxon test- MASS

	PorgA_EC1	PorgA_EC1_F1	PorgA_EC1_F2	PorgA_EC2	PorgA_EC2_F1	PorgA_EC2_F2	PorgB_EC1	PorgB_EC1_F1	PorgB_EC1_F2	PorgB_EC2	PorgB_EC2_F1
PorgA_EC1_F1	0.0475	-	-	-	-	-	-	-	-	-	-
PorgA_EC1_F2	1.0000	1.0000	-	-	-	-	-	-	-	-	-
PorgA_EC2	0.0067	1.0000	1.0000	-	-	-	-	-	-	-	-
PorgA_EC2_F1	5.0e-05	0.0222	0.0023	0.0679	-	-	-	-	-	-	-
PorgA_EC2_F2	0.0018	1.0000	1.0000	1.0000	0.1303	-	-	-	-	-	-
PorgB_EC1	1.5e-05	0.1239	0.0047	0.3642	1.0000	0.8276	-	-	-	-	-
PorgB_EC1_F1	0.0050	1.0000	1.0000	1.0000	0.0610	1.0000	0.3906	-	-	-	-
PorgB_EC1_F2	0.0011	1.0000	0.2531	1.0000	1.0000	1.0000	1.0000	1.0000	-	-	-
PorgB_EC2	1.0000	1.0000	1.0000	1.0000	0.0028	1.0000	0.0057	1.0000	0.1771	-	-
PorgB_EC2_F1	1.9e-06	0.1222	0.0038	0.7090	1.0000	0.7001	1.0000	0.4200	1.0000	0.0029	-
PorgB_EC2_F2	0.0028	1.0000	1.0000	1.0000	0.4062	1.0000	0.5966	1.0000	1.0000	1.0000	0.7873

HP – Trial 2018-04-26- Po B & Po A – Wilcoxon test- DIAMETER

	PorgA_EC1	PorgA_EC1_F1	PorgA_EC1_F2	PorgA_EC2	PorgA_EC2_F1	PorgA_EC2_F2	PorgB_EC1	PorgB_EC1_F1	PorgB_EC1_F2	PorgB_EC2	PorgB_EC2_F1
PorgA_EC1_F1	0.00022	-	-	-	-	-	-	-	-	-	-
PorgA_EC1_F2	0.15666	1.00000	-	-	-	-	-	-	-	-	-
PorgA_EC2	1.9e-05	1.00000	1.00000	-	-	-	-	-	-	-	-
PorgA_EC2_F1	5.2e-06	0.02527	0.00256	0.17965	-	-	-	-	-	-	-
PorgA_EC2_F2	2.2e-06	1.00000	1.00000	1.00000	0.37572	-	-	-	-	-	-
PorgB_EC1	7.0e-07	9.5e-06	2.4e-06	3.7e-05	0.36117	5.1e-05	-	-	-	-	-
PorgB_EC1_F1	2.2e-08	5.6e-06	7.7e-07	6.3e-05	1.00000	9.0e-05	1.00000	-	-	-	-
PorgB_EC1_F2	4.6e-07	0.00313	0.00026	0.02676	1.00000	0.05973	1.00000	1.00000	-	-	-
PorgB_EC2	5.2e-07	0.11444	0.01459	1.00000	1.00000	1.00000	0.00829	0.24040	1.00000	-	-
PorgB_EC2_F1	4.1e-08	0.00065	0.00012	0.00727	1.00000	0.01202	1.00000	1.00000	1.00000	1.00000	-
PorgB_EC2_F2	3.3e-08	0.00788	0.00243	0.10649	1.00000	0.28264	0.01503	0.15213	1.00000	1.00000	1.00000

HP – Trial 2018-06-21 – Po B – Kruskal-Wallis and Wilcoxon test - DIAMETER

	M0	M0CT	M1	M1CT	M2
M0CT	1.00000	-	-	-	-
M1	1.00000	1.00000	-	-	-
M1CT	0.00355	0.00571	0.00069	-	-
M2	0.69557	0.58979	0.56286	1.00000	-
M2CT	0.77167	0.65720	0.14562	1.00000	1.00000

HP – Trial 2018-08-31 – Po B – Welch ANOVA & Games Howell tests – MASS

	p
M0CT-M0	.35
M1-M0	.004
M1CT-M0	.961
M2-M0	.997
M2CT-M0	.76
M3-M0	1
M3CT-M0	.892
M4-M0	1
M4CT-M0	.795
M1-M0CT	.988
M1CT-M0CT	.986
M2-M0CT	.116
M2CT-M0CT	1
M3-M0CT	.369
M3CT-M0CT	.995
M4-M0CT	.901
M4CT-M0CT	1
M1CT-M1	.382
M2-M1	.002
M2CT-M1	.672
M3-M1	.011
M3CT-M1	.433
M4-M1	.392
M4CT-M1	.957
M2-M1CT	.65
M2CT-M1CT	1
M3-M1CT	.948
M3CT-M1CT	1
M4-M1CT	1
M4CT-M1CT	1
M2CT-M2	.35
M3-M2	1
M3CT-M2	.495
M4-M2	.998
M4CT-M2	.423
M3-M2CT	.75
M3CT-M2CT	1
M4-M2CT	.992
M4CT-M2CT	1
M3CT-M3	.876
M4-M3	1
M4CT-M3	.773
M4-M3CT	.998
M4CT-M3CT	1
M4CT-M4	.985

HP – Trial 2018-08-31 – Po B – Kruskal Wallis & Wilcoxon tests – DIAMETER

	M0	M0CT	M1	M1CT	M2	M2CT	M3	M3CT	M4
M0CT	1.00000	-	-	-	-	-	-	-	-
M1	0.00072	0.22392	-	-	-	-	-	-	-
M1CT	1.00000	1.00000	0.02019	-	-	-	-	-	-
M2	1.00000	0.01447	4.3e-06	0.03970	-	-	-	-	-
M2CT	1.00000	0.38042	0.00369	1.00000	1.00000	-	-	-	-
M3	0.49652	0.00319	9.9e-07	0.00743	1.00000	1.00000	-	-	-
M3CT	0.34344	0.00222	2.7e-05	0.00664	1.00000	1.00000	1.00000	-	-
M4	1.00000	0.18870	0.01589	0.76816	1.00000	1.00000	1.00000	1.00000	-
M4CT	1.00000	1.00000	0.02111	1.00000	1.00000	1.00000	1.00000	0.53839	1.00000

- **L-SSF and L-digestate: p-values**

HP – Trial 2018-04-26- L-digestate - ANOVA & Tukey HSD test- MASS

	p
EC1_F1-EC1	1
EC1_F2-EC1	.99
EC2-EC1	.942
EC2_F1-EC1	.999
EC2_F2-EC1	.035
EC1_F2-EC1_F1	.944
EC2-EC1_F1	.988
EC2_F1-EC1_F1	1
EC2_F2-EC1_F1	.013
EC2-EC1_F2	.643
EC2_F1-EC1_F2	.929
EC2_F2-EC1_F2	.155
EC2_F1-EC2	.994
EC2_F2-EC2	.002
EC2_F2-EC2_F1	.013

HP – Trial 2018-06-21 – L-digestate – Kruskal-Wallis and Wilcoxon test - MASS

	M0	M0CT	M1	M1CT	M2
M0CT	0.061	-	-	-	-
M1	0.994	1.000	-	-	-
M1CT	0.031	1.000	1.000	-	-
M2	0.412	1.000	1.000	1.000	-
M2CT	0.147	1.000	1.000	1.000	1.000

HP – Trial 2018-06-21 – L-SSF – Kruskal-Wallis and Wilcoxon test – MASS

	M0	M0CT	M1	M1CT	M2
M0CT	1.00000	-	-	-	-
M1	1.6e-06	0.01126	-	-	-
M1CT	5.4e-09	3.3e-05	0.07449	-	-
M2	1.3e-07	0.00235	1.00000	0.89911	-
M2CT	4.9e-10	0.00025	0.02603	1.00000	1.00000

HP – Trial 2018-06-21 – L-SSF – Kruskal-Wallis and Wilcoxon test – DIAMETER

	M0	M0CT	M1	M1CT	M2
M0CT	1.0000	-	-	-	-
M1	8.8e-07	0.0239	-	-	-
M1CT	2.8e-09	1.9e-05	0.0259	-	-
M2	9.3e-08	0.0012	1.0000	1.0000	-
M2CT	0.0447	1.0000	1.0000	0.1351	0.9279

HP – Trial 2018-08-31- L-digestate – Welch ANOVA & Games Howell tests – MASS

	p
M1-M0	.549
M1HA-M0	.981
M2-M0	.004
M2HA-M0	.958
M3-M0	.114
M3HA-M0	1
M4-M0	.103
M4HA-M0	1
M5-M0	.654
M5HA-M0	1
M1HA-M1	1
M2-M1	.138
M2HA-M1	1
M3-M1	.992
M3HA-M1	.677
M4-M1	.951
M4HA-M1	.598
M5-M1	1
M5HA-M1	.799
M2-M1HA	.28
M2HA-M1HA	1
M3-M1HA	.992
M3HA-M1HA	.966
M4-M1HA	.963
M4-M1HA	.963
M4HA-M1HA	.987
M5-M1HA	1
M5HA-M1HA	.999
M2HA-M2	.209
M3-M2	.403
M3HA-M2	.015
M4-M2	.818
M4HA-M2	.005
M5-M2	.499
M5HA-M2	.008
M3-M2HA	.987
M3HA-M2HA	.943
M4-M2HA	.945
M4HA-M2HA	.97
M5-M2HA	1
M5HA-M2HA	.997
M3HA-M3	.292
M4-M3	1
M4HA-M3	.131
M5-M3	1
M5HA-M3	.194
M4-M3HA	.224
M4HA-M3HA	1
M5-M3HA	.695
M5HA-M3HA	.999
M4HA-M4	.119
M5-M4	.999
M5HA-M4	.19
M5-M4HA	.695
M5HA-M4HA	1
M5HA-M5	.863

HP – Trial 2018-08-31- L-digestate – Kurkal Wallis & Wilcoxon tests – DIAMETER

	M0	M1	M1HA	M2	M2HA	M3	M3HA	M4	M4HA	M5
M1	1.0000	-	-	-	-	-	-	-	-	-
M1HA	1.0000	0.3282	-	-	-	-	-	-	-	-
M2	0.1148	1.0000	0.0073	-	-	-	-	-	-	-
M2HA	1.0000	1.0000	1.0000	0.0922	-	-	-	-	-	-
M3	1.0000	1.0000	0.1915	0.7668	1.0000	-	-	-	-	-
M3HA	1.0000	1.0000	1.0000	0.1474	1.0000	1.0000	-	-	-	-
M4	1.0000	1.0000	1.0000	0.2032	1.0000	1.0000	1.0000	-	-	-
M4HA	1.0000	0.0171	1.0000	5.2e-05	1.0000	0.0036	1.0000	0.3808	-	-
M5	1.0000	1.0000	1.0000	0.0096	1.0000	1.0000	1.0000	1.0000	1.0000	-
M5HA	1.0000	1.0000	1.0000	0.0141	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

ANNEX 2: P-VALUES FOR STATISTICAL TESTS FOR SOLID FERTILIZERS REPORT

- **Basil harvest**

Harvest 1: Kruskal Wallis & Wilcoxon tests

	B1-L-D	B10-S-Te	B2-L-VC	B3-L-JC	B4-L-DV	B5-F-D	B6-F-VC	B7-F-To	B8-S-D
B10-S-Te	0.0021	-	-	-	-	-	-	-	-
B2-L-VC	0.0811	0.0016	-	-	-	-	-	-	-
B3-L-JC	0.0208	0.0016	0.0016	-	-	-	-	-	-
B4-L-DV	1.0000	0.0062	0.0016	0.0016	-	-	-	-	-
B5-F-D	0.0088	1.0000	0.0016	0.0016	0.0154	-	-	-	-
B6-F-VC	1.0000	0.0043	0.0021	0.0016	1.0000	0.0811	-	-	-
B7-F-To	0.0021	1.0000	0.0016	0.0016	0.0110	1.0000	0.0212	-	-
B8-S-D	0.0043	0.6334	0.0016	0.0016	0.0402	1.0000	0.0723	1.0000	-
B9-S-VC	0.0259	0.0016	0.0031	0.0020	0.0016	0.0016	0.0016	0.0016	0.0016

Harvest 2: Kruskal Wallis & Wilcoxon tests

	B1-L-D	B10-S-Te	B2-L-VC	B3-L-JC	B4-L-DV	B5-F-D	B6-F-VC	B7-F-To	B8-S-D
B10-S-Te	1.0000	-	-	-	-	-	-	-	-
B2-L-VC	0.0069	0.0492	-	-	-	-	-	-	-
B3-L-JC	0.0016	0.0021	0.0190	-	-	-	-	-	-
B4-L-DV	0.0055	0.0264	1.0000	0.0069	-	-	-	-	-
B5-F-D	1.0000	1.0000	0.0044	0.0016	0.0016	-	-	-	-
B6-F-VC	3.3e-05	0.0024	0.0987	1.0000	0.0088	3.3e-05	-	-	-
B7-F-To	0.0016	0.0026	0.2466	0.4858	0.0067	0.0016	1.0000	-	-
B8-S-D	0.4212	1.0000	1.0000	0.0077	1.0000	0.0823	0.0295	0.2069	-
B9-S-VC	0.0016	0.0049	1.0000	0.2457	0.0894	0.0016	1.0000	1.0000	0.3548

Cumulative harvest: Kruskal Wallis & Wilcoxon tests

	B1-L-D	B10-S-Te	B2-L-VC	B3-L-JC	B4-L-DV	B5-F-D	B6-F-VC	B7-F-To	B8-S-D
B10-S-Te	1.00000	-	-	-	-	-	-	-	-
B2-L-VC	0.00023	0.00023	-	-	-	-	-	-	-
B3-L-JC	0.00164	0.00164	0.00386	-	-	-	-	-	-
B4-L-DV	0.00874	0.00874	1.00000	0.00162	-	-	-	-	-
B5-F-D	1.00000	1.00000	3.3e-05	0.00164	0.00163	-	-	-	-
B6-F-VC	0.00163	0.00163	1.00000	0.00341	0.05976	0.00163	-	-	-
B7-F-To	0.00880	0.00343	1.00000	0.00163	1.00000	0.00164	0.04023	-	-
B8-S-D	1.00000	0.80556	0.01923	0.00164	0.38445	0.58602	0.00268	0.04965	-
B9-S-VC	0.00164	0.00164	1.00000	0.01719	0.00432	0.00164	0.93646	0.00696	0.00185

- **Number of tomatoes**

Number of ripe tomatoes per plant – ANOVA & Tukey HSD tests

	p
B2-B1	1
B3-B1	1
B4-B1	.934
B5-B1	.001
B6-B1	.854
B7-B1	.862
B8-B1	<.001
B9-B1	.063
B10-B1	<.001
B3-B2	1
B4-B2	.991
B5-B2	.001
B6-B2	.967
B7-B2	.97
B8-B2	<.001
B9-B2	.128
B10-B2	<.001
B4-B3	.934
B5-B3	.001
B6-B3	.854
B7-B3	.862
B8-B3	<.001
B9-B3	.063
B10-B3	<.001
B5-B4	.011
B6-B4	1
B7-B4	1
B8-B4	<.001
B9-B4	.552
B10-B4	<.001
B6-B5	.017
B7-B5	.016
B8-B5	.033
B9-B5	.496
B10-B5	.575
B7-B6	1
B8-B6	<.001
B9-B6	.687
B10-B6	<.001
B8-B7	<.001
B9-B7	.676
B10-B7	<.001
B9-B8	<.001
B10-B8	.791
B10-B9	.011

Number of ripe and green tomatoes per plant – ANOVA & Tukey HSD tests

	p
B2-B1	1
B3-B1	.993
B4-B1	1
B5-B1	<.001
B6-B1	1
B7-B1	1
B8-B1	<.001
B9-B1	.396
B10-B1	<.001
B3-B2	1
B4-B2	.979
B5-B2	<.001
B6-B2	.988
B7-B2	1
B8-B2	<.001
B9-B2	.162
B10-B2	<.001
B4-B3	.885
B5-B3	<.001
B6-B3	.918
B7-B3	.996
B8-B3	<.001
B9-B3	.08
B10-B3	<.001
B5-B4	<.001
B6-B4	1
B7-B4	1
B8-B4	<.001
B9-B4	.714
B10-B4	<.001
B6-B5	<.001
B7-B5	<.001
B8-B5	1
B9-B5	<.001
B10-B5	.913
B7-B6	1
B8-B6	<.001
B9-B6	.657
B10-B6	<.001
B8-B7	<.001
B9-B7	.355
B10-B7	<.001
B9-B8	<.001
B10-B8	.767
B10-B9	<.001

Number of ripe and green tomatoes per plant and flowers– ANOVA & Tukey HSD tests

	p
B2-B1	1
B3-B1	.448
B4-B1	.693
B5-B1	<.001
B6-B1	.472
B7-B1	.243
B8-B1	.001
B9-B1	1
B10-B1	.001
B3-B2	.602
B4-B2	.833
B5-B2	<.001
B6-B2	.629
B7-B2	.36
B8-B2	<.001
B9-B2	.998
B10-B2	<.001
B4-B3	1
B5-B3	<.001
B6-B3	1
B7-B3	1
B8-B3	<.001
B9-B3	.2
B10-B3	<.001
B5-B4	<.001
B6-B4	1
B7-B4	.997
B8-B4	<.001
B9-B4	.377
B10-B4	<.001
B6-B5	<.001
B7-B5	<.001
B8-B5	1
B9-B5	.001
B10-B5	1
B7-B6	1
B8-B6	<.001
B9-B6	.215
B10-B6	<.001
B8-B7	<.001
B9-B7	.093
B10-B7	<.001
B9-B8	.002
B10-B8	1
B10-B9	.002

- **Mass of tomatoes:** Kruskal Wallis & Wilcoxon tests

	B1	B2	B3	B4	B5	B6	B7	B8	B9
B2	1.00000	-	-	-	-	-	-	-	-
B3	1.00000	1.00000	-	-	-	-	-	-	-
B4	0.02512	1.5e-06	1.00000	-	-	-	-	-	-
B5	1.5e-05	8.4e-14	0.00450	3.0e-06	-	-	-	-	-
B6	1.00000	1.00000	1.00000	0.00022	< 2e-16	-	-	-	-
B7	1.00000	1.00000	1.00000	0.00721	< 2e-16	1.00000	-	-	-
B8	1.00000	0.00036	0.00090	< 2e-16	< 2e-16	1.1e-13	3.3e-13	-	-
B9	1.00000	5.3e-08	4.4e-06	< 2e-16	< 2e-16	< 2e-16	< 2e-16	0.09745	-
B10	1.00000	0.03202	0.00668	< 2e-16	< 2e-16	5.5e-10	4.3e-10	1.00000	0.26704

- **Yield of tomato plants**

	p
B2-B1	1
B3-B1	1
B4-B1	1
B5-B1	.525
B6-B1	.989
B7-B1	.99
B8-B1	<.001
B9-B1	.047
B10-B1	<.001
B3-B2	1
B4-B2	1
B5-B2	.895
B6-B2	1
B7-B2	1
B8-B2	<.001
B9-B2	.219
B10-B2	<.001
B4-B3	.999
B5-B3	.467
B6-B3	.979
B7-B3	.981
B8-B3	<.001
B9-B3	.038
B10-B3	<.001
B5-B4	.859
B6-B4	1
B7-B4	1
B8-B4	<.001
B9-B4	.145
B10-B4	<.001
B6-B5	.973
B7-B5	.971
B8-B5	<.001
B9-B5	.892
B10-B5	.002
B7-B6	1
B8-B6	<.001
B9-B6	.281
B10-B6	<.001
B8-B7	<.001
B9-B7	.274
B10-B7	<.001
B9-B8	.001
B10-B8	.87
B10-B9	.035

- **Height of tomato plants**

	p
B2-B1	.979
B3-B1	.653
B4-B1	1
B5-B1	1
B6-B1	.979
B7-B1	.015
B8-B1	.486
B9-B1	.046
B10-B1	.336
B3-B2	.997
B4-B2	1
B5-B2	.921
B6-B2	1
B7-B2	.135
B8-B2	.08
B9-B2	.004
B10-B2	.046
B4-B3	.921
B5-B3	.486
B6-B3	.997
B7-B3	.486
B8-B3	.015
B9-B3	.001
B10-B3	.008
B5-B4	.997
B6-B4	1
B7-B4	.046
B8-B4	.218
B9-B4	.015
B10-B4	.135
B6-B5	.921
B7-B5	.008
B8-B5	.653
B9-B5	.08
B10-B5	.486
B7-B6	.135
B8-B6	.08
B9-B6	.004
B10-B6	.046
B8-B7	<.001
B9-B7	<.001
B10-B7	<.001
B9-B8	.921
B10-B8	1
B10-B9	.979

ANNEX 3: PHOTO OF BASIL HARVEST 1 – FOR SOLID FERTILIZERS REPORT

- Trial “Lasagna bed”: B1-L-D, B2-L-VC, B3-L-JC, B4-L-DV

- Trial “Fertilizer mix”: B5-F-D, B6-F-VC, B7-F-To

- Trial “Substrate”: B8-S-D, B9-S-VC, B10-S-Te

ANNEX 4: PHOTO OF BASIL HARVEST 2 – FOR SOLID FERTILIZERS REPORT

- Trial “Lasagna bed”: B1-L-D, B2-L-VC, B3-L-JC, B4-L-DV

- Trial “Fertilizer mix”: B5-F-D, B6-F-VC, B7-F-To

- Trial “Substrate”: B8-S-D, B9-S-VC, B10-S-Te

ANNEX 5: PHOTO OF BASIL BEFORE HARVEST 3 – FOR SOLID FERTILIZERS

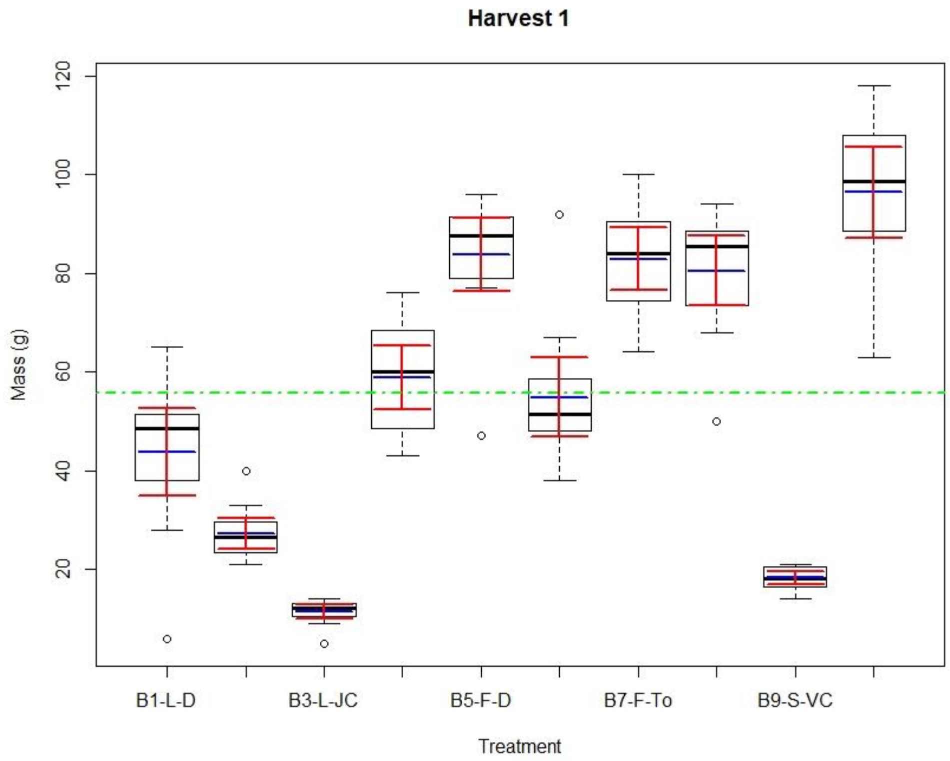
- Trial “Lasagna bed”: B1-L-D, B2-L-VC, B3-L-JC, B4-L-DV

- Trial “Fertilizer mix”: B5-F-D, B6-F-VC, B7-F-To

- Trial “Substrate”: B8-S-D, B9-S-VC, B10-S-Te

ANNEX 6: RESULTS FOR BASIL FIRST HARVEST FOR SOLID FERTILIZERS REPORT

Harvest 1 of Basil in Container trial



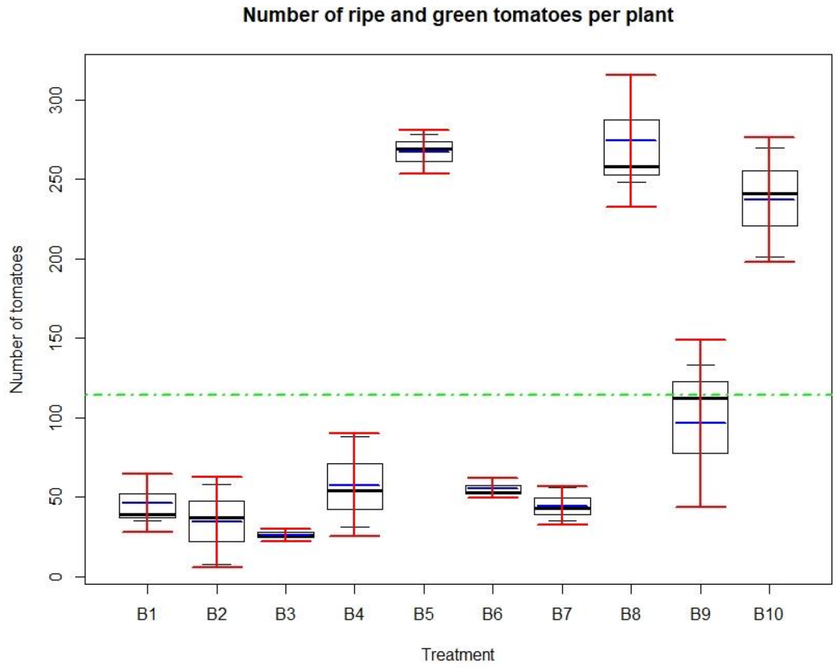
- "Lasagna bed": B3 gave mass significantly smaller than all the other treatments. And B4 gave mass significantly higher than B2.
- "Fertilizer mix": B7 gave mass significantly higher than B6.
- "Substrate": B8 and B10 gave yield higher than B9.

Figure 40: Harvest 1 – Fresh mass of basil

Concerning the first harvest, the treatments containing S-digestate were among those that gave highest mass of basil harvested. On the contrary, the treatments that gave the lowest mass of basil were containing compost.

ANNEX 7: RESULTS FOR TOTAL NUMBER OF TOMATO FRUITS FOR SOLID FERTILIZERS REPORT WITH AND WITHOUT FLOWERS

Number of ripe and green tomatoes



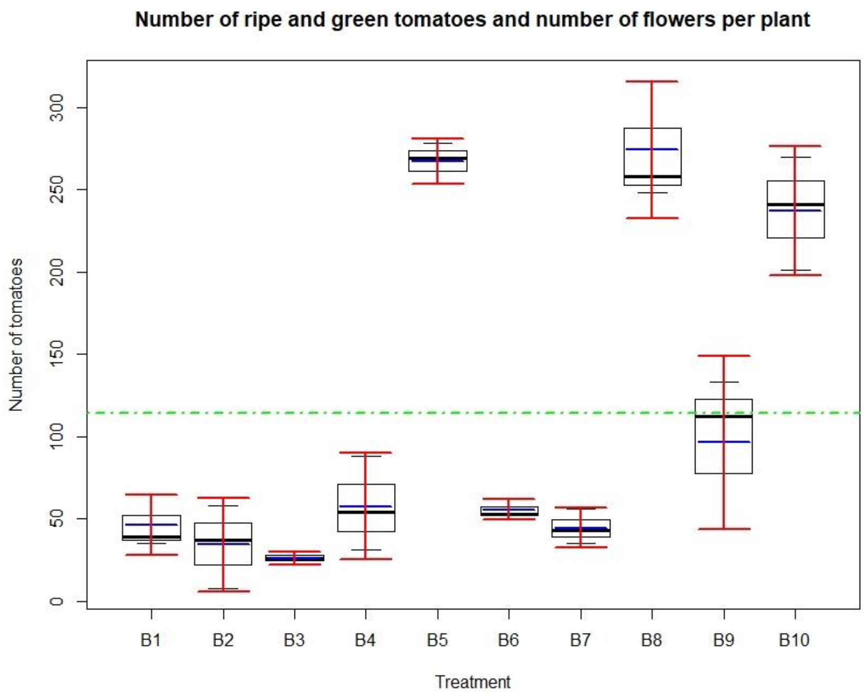
- “Lasagna bed”: no significant difference between B1, B2, B3 and B4.
- “Fertilizer mix”: B5 gave number of tomatoes per plant significantly higher than B6 and B7.
- “Substrate”: B8 gave number of tomatoes per plant significantly higher than B9.

Figure 41: Number of ripe and green tomatoes per plant

In conclusion, including green tomatoes in the analysis of the number of tomatoes per plants does not alter the results found by modelling the number of ripe tomatoes only.

In the next paragraph, the analysis includes also the number of flowers collected at the end of the field test, as it represents the potential number of future fruits.

Number of ripe and green tomatoes and number of flowers



- “Lasagna bed”: no significant difference between B1, B2, B3 and B4.
- “Fertilizer mix”: B5 gave number of tomatoes per plant significantly higher than B6 and B7.
- “Substrate”: B8 gave number of tomatoes per plant significantly higher than B9.

Figure 42: Number of ripe and green tomatoes and flowers per plant

In conclusion, including number of flowers in the analysis of the number of tomatoes per plants led to the same results found when investigating the number of tomatoes only.

ANNEX 8: STATISTICAL TESTS FOR BIOPESTICIDE REPORT

- **P-values**

Bt – Trial 3 – Bt SSF and control – Pearson's Chi-squared test

```
        Pearson's Chi-squared test with Yates' continuity correction

data:  d
X-squared = 7.3717, df = 1, p-value = 0.006626
```

Bt – Trial 3 – Bt SSF and Bt commercial – Fisher's Exact test

```
        Fisher's Exact Test for Count Data

data:  d
p-value = 4.115e-08
```

Bt – Trial 3 – Bt commercial and control – Fisher's Exact test

```
        Fisher's Exact Test for Count Data

data:  d
p-value = 7.419e-16
```

Bt – Trial 4 – Cabbages - Bt SSF and control – Pearson's Chi-squared test

```
        Pearson's Chi-squared test with Yates' continuity correction

data:  d
X-squared = 61.06, df = 1, p-value = 5.537e-15
```

Bt – Trial 4 – Cabbages – Bt SSF and Bt commercial – Fisher's Exact test

```
        Fisher's Exact Test for Count Data

data:  d
p-value = 0.0001522
```

Bt – Trial 4 – Cabbages – Bt commercial and control – Fisher's Exact test

```
        Fisher's Exact Test for Count Data

data:  d
p-value < 2.2e-16
```

Bt – Trial 4 – Trays - Bt SSF and control – Pearson's Chi-squared test

```
        Pearson's Chi-squared test with Yates' continuity correction

data:  d
X-squared = 9.433, df = 1, p-value = 0.002131
```

Bt – Trial 4 – Trays – Bt SSF and Bt commercial – Fisher's Exact test

```
        Fisher's Exact Test for Count Data

data:  d
p-value = 4.236e-11
```

Bt – Trial 4 – Trays – Bt commercial and control – Fisher's Exact test

```
        Fisher's Exact Test for Count Data

data:  d
p-value < 2.2e-16
```

- **Results and confidence interval**

	L-SSF and control	Confidence interval	L-SSF and Commercial Bt	Confidence interval	Commercial Bt and control	Confidence interval
Trial 3	+23 %	(0.08; 0.38)	-26 %	(-0.36; -0.16)	+49 %	(0.60; 0.37)
Trial 4-Cabbage	+76 %	(0.64; 0.88)	-20 %	(-0.31; -0.09)	+96 %	(1;0.91)
Trial 4-Trays	+2 4%	(0.1; 0.38)	-33 %	(-0.43; -0.24)	+57 %	(0.68;0.47)

Contact

Bérengère Duval
berengere.duval@refarmers.co
Refarmers
13 avenue de Verdun
69130 Ecully
France