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Effects of Different Substrates on Basil Cultivation

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Abstract

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This project was part of the 6Aika: CircularHoodFood project and was funded by EAKR Uudenmaan liitto. The project was carried out in Metropolia University of Applied Sciences, UrbanFarmlab.

The main goal of this project was to examine effects of different substrates on quantitative properties of basils and ethereal oil composition of basil leaves. LECA, perlite, cellulose fibre and recycled absorbent cloth were examined as substrates. Basils were cultivated under optimized greenhouse conditions.

The project consisted of a four-week cultivation period alongside various analyses. These analyses were pH and electric conductivity, nitrogen and phosphorus analyses, qualitative ethereal oil analyses and measurements regarding the growth of basils.

According to qualitative ethereal oil analyses, each basil biosynthesised linalool, eugenol and eucalyptol. Methyl eugenol either was not biosynthesised at all or had little area percentage compared to other three ethereal oils. When the substrates and basil plants were observed during the cultivation period, mould contamination on cellulose fibre and textile fibre was noticed. The basils grown on these had signs of necrosis on the lower leaves, while basils grown on perlite and LECA were healthier. However, basils grown on both perlite and cellulose fibre had better crop properties (mass, height and leaf quantity) than textile fibre and LECA. All in all, when both observational results and crop results were compiled, perlite was the sole satisfactory alternative among the four substrates.

Results of this project offer the idea of substituting the use of peat, which will be prohibited in soilless or urban farming in Finland, with alternative substrates.

Keywords: hydroponic system, ethereal oil, basil, soilless, GC-MS, urban farming

Tiivistelmä

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Tämän projektin rahoitti EAKR Uudenmaan liitto. Sen lisäksi, tämä projekti oli osa 6Aika:circularHoodFood hanketta. Projekti toteutettiin Metropolia Ammattikorkelkoulun UrbanFarmLab-tilassa.

Projektin päätavoitteena oli tutkia erilaisten kasvatusalustojen vaikutuksia basilikan satoon ja eteeristen öljyjen koostumukseen. Kasvualustoina käytettiin Leca-soraa, perliittiä, sellusilppua ja kierrätettyä tekstiilikuitua. Basilikoita kasvatettiin optimoiduissa kasvihuoneolosuhteissa.

Kasvatuskoe kesti neljä viikkoa ja tämän jälkeen tehtiin erilaisia analyysejä. Näitä analyysejä olivat pH ja sähkönjohtavuus mittaus, typpi- ja fosforianalyysit, eteeristen öljyjen analyysit sekä sadon saantoa koskevat mittaukset.

Kvalitatiivisten eteeristen öljyjen analyysien perusteella kaikki basilikat muodostivat linalolia, eugenolia ja eukalyptolia. Metyylieugenolia ei muodostunut kaikissa basilikoissa. Sellusilpussa ja tekstiilikuidussa havaittiin homekontaminaatiota kasvualustojen ja basilikojen kuntojen tarkasteluissa. Sen lisäksi basilikoiden alalehtiin ilmestyi nekroosia, toisaalta perliitissä ja Leca-sorassa kasvaneet basilikat olivat terveempiä. Kuitenkin sekä perliitillä että sellusilpulla kasvatettujen basilikoiden sato-ominaisuudet (massa, pituus ja lehtimäärät) olivat kuitenkin paremmat kuin tekstiilikuidulla ja Lecalla. Kaiken kaikkiaan, kun sekä havainnointitulokset että satotulokset koottiin yhteen, perliitti oli ainoa tyydyttävä vaihtoehto neljästä kasvualustasta.

Projektin tulokset tarjoavat mahdollisuuden korvata turpeen käytön vaihtoehtoisilla kasvatusalustalla, koska turpeen käyttö tullaan kieltämään vesi- ja kaupunkiviljelyssä Suomessa.

Avainsanat: vesiviljelysysteemi, eteeriset öljyt, basilika, GC-MS, urbaaniviljely

Contents

List of Abbreviations

EC: Electrical conductivity

HS-GC-MS: Head-Space Gas chromatography-mass spectrometry, device to analyse ethereal oils

LECA: Lightweight expanded clay aggregate, a type of substrate used in hydroponic agriculture

DXP: 1-deoxy-*D*-xylulose-5-phosphate, an enzyme used by plants in synthetisisation of ethereal oils

MEP: 2C-methyl-*D*-erythritol-4-phosphate, an enzyme used by plants in synthetisisation of ethereal oils

1 Introduction

Hydroculture and hydroponics, even though already being used by ancient civilizations, like the hanging gardens of Babylon or the floating garden of Aztecs, is the future of farming. Although there are quite a fascinating number of examples of earlier versions of soilless growing in history, rapid development of new technologies for soilless growing is quite new compared to human history. In addition, most of the human population was and is still fed on classical agriculture crops.

While humanity grows in exponential rate and the Earth cannot offer infinite space of land to convert into farming fields, hydroculture requires less space to work with and the final product is identical. Nonetheless, soilless growing with the addition of vertical farming or rooftop farming, both which are possible and practiced in some ways in urban life under the name of urban farming, can expand the crop amount as required.

Moreover, soilless growing has less impact on environment than classical agriculture in different ways. The most common examples are the water sources becoming polluted by washed up fertilizers and pesticides or forests felled for more farming fields. On the other hand, soilless growing is a closed system; therefore, waste management is unchallenging to take under control and does not require as large space as classical agriculture.

Thus, soilless agriculture still requires development and research projects. With newer technology and knowledge soilless agriculture is becoming more efficient and have less environmental impact.

1.1 Project Goals

This project was part of the 6Aika: CircularHoodFood project and was funded by EAKR Uudenmaan liitto. This project was executed for Metropolia University of Applied Sciences' UrbanFarmLab project. Its goal was to examine whether

different substrates have any effect on basil cultivation in terms of crops quantity and ethereal oils composition or if all substrates, which are suitable for basil cultivation, give the same results. LECA, perlite, cellulose fibres and recycled textile fibres are the examined substrates. The project was carried out under controlled conditions in greenhouse in Metropolia's UrbanFarmLab space.

1.2 Documentation

Through the project Microsoft Teams was used as the main application for storing meeting notes, reports, analyses data, and pictures. Furthermore, a backup folder was saved regularly to OneDrive, in case Microsoft Teams group becomes unreachable or the documents were deleted unintentionally. A follow-up logbook was updated during the project to record daily differences of basil plants and substrates.

2 Drip Irrigation

Drip irrigation systems are one of the six types of hydroponic system utilized today. To slow down the flow of dripped nutrient solution, moderately absorbent growing medium is used for planting [1].

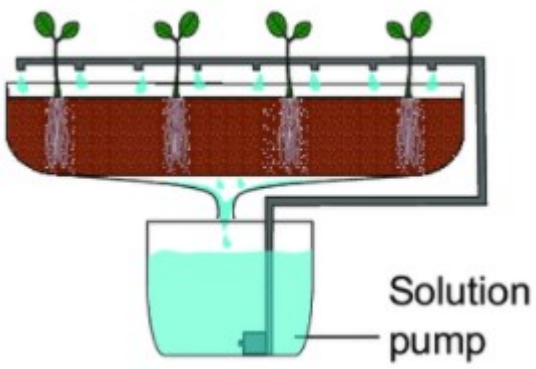

Figure 1. Simple illustration of a drip irrigation system [2.] Adapted from "Getting to the roots of aeroponic indoor farming", by Bethany May Eldridge; Lillian R. Manzoni; Calum A. Graham & Billy Rodgers, 2020, New Phytologist, 228, p. 1184. CC BY.

Dripping irrigation systems work with a relatively high pressure at the pump via microtubing and drip emitters. At the emitters, the pressure is reduced to uniform lower pressure. Every plant receives the equal amount of delivery of nutrient solution by this method. [3, p. 163.] Nutrient solution flows through the substrate, the flow rate depends on the substrate used, and is drained out of the substrate. Finally drained nutrient solution is collected back in the water tank and is recirculated in the system like in Figure 1. This reduces the water consumption considerably.

While collected water have significant advantages, several liabilities of recirculating the water are quite dangerous for cultivated plants health. Autotoxicity is one of the liabilities. Secreted chemicals, like organic acids, amino acids sugars, from the roots into the nutrient solution. Accumulated chemicals may cause reduction of the yield as an example. [4.] Micro-organisms spreading through the recirculating nutrient solution is another risk of a closed hydroponic system.

3 Plant Cultivation

There are six main key factors in plant cultivation. These factors are

- light
- temperature
- relative humidity
- CO_2 (carbon dioxide)
- irrigation
- fertilization. [5.]

Values of these factors differ for every plant; thus, the requirements of basil are focused on.

Light's intensity, direction and wavelength alters how light affects the plant. Plants' photosynthesis rate, growth and yield capacity depends on the light. 400–700 nm of wavelength is critical for photosynthesis. [5, p. 54.] According to

Putievsky and Galambosi [6], long periods of lighting is perfect for basil cultivation and basil can take up to 24 hours of light with top yield as result. Direction of light mainly effects the activation of the phytochrome proteins. These proteins are largely locating around the growing point and nodes. Phytochrome and various hormones of the plants have an impact on the way plant grows and developmental rhythm. [5, p. 58.]

Photosynthesis, plant respiration and nutrient solution absorption is influenced by temperature. The high temperature increases the growth rate of the plants, unless the temperature exceeds the protein's deactivation limit or plants' leaves respire or evaporation is too great to cause plants to wither. [5, p. 48–50.] According to Putievsky and Galambosi [6], basil's growth rate and quantity of a harvest were at peak at a temperature of 27 °C.

Water flow inside a plant is essential, considering water carries, for example nutrients, enzymes, proteins to each part of plant [7]. To maintain the water flow plants let water vaporize from the stomata scattered all over the abaxial surface of leaves. This assists the plant to cool. High saturation deficit is required to achieve water vaporization; nevertheless, when the saturation deficit is too high, it causes water vaporization rate to increase and stomata is closed to avoid losing water. [5, p. 66–67.]

Plants need carbon dioxide entirely for photosynthesis. CO_2 is the limiting factor. The lower limit of CO_2 for plants standard growth is around 200 ppm. The air circulation is essential to transport CO_2 towards the stomata for a fluent plant respiration. [5, p. 83–85.]

Plants cannot function without water. Water is utilized as the key material of metabolism and photosynthesis of plants, as the solvent for water-soluble materials and as a physical support by means of turgor pressure. Water (including nutrient substances) is absorbed through roots, utilizing the principle of osmosis. Osmosis is weakened when the solid concentration of water is high, which causes water deficiency. Water deficiency is revealed as withering of the plant while the vital functions of the plant are decelerated. [5, p. 74–78.]

Putievsky and Galambosi report that continues water supply is required in basil cultivation, yet basil is intolerant to water stress. 22% to 41% decrease in the dry weight of leaves is caused by mild and moderate water stress, whereas 87% to 100% increase in ethereal oil content is caused by water stress. [6.]

Fertilizers are the nutrient supply of plants. For plants, the nutrients are elements that are necessary for photosynthesis and used as components of organs of plants. Nutrients are divided into two groups by the level of demand: macronutrients and micronutrients. To date, there are total of 16 nutrient elements. The nutrients are absorbed through roots alongside water. Nearly all nutrients occur in cationic form. [5, p. 79–82.]

4 Substrates

Substrates, in terms of hydroponic systems, are substitutes for soil. Oxygen and support for roots are provided by substrates. [8, p. 89.] Numerous substrate candidates are eliminated by similar other projects, considering that these candidates were for instance not sustainable, excessively waterlogged or dehydrated [9, p. 80].

For acceptable water retention porous materials and small particle-size are recommended, thus water is stored well within particles. The substrates containing components such as sodium chloride or calcium carbonate is accounted as toxic material to plants as these particles tend to separate from the substrate. This causes alterations in salt concentration and pH level. [8, p. 89–90.]

Substrates can be divided into two categories: Organic and inorganic materials. Each and every substrate with organic chemical properties are included in the organic materials category. Decomposition rate of organic materials with the assistance of nutrient solution are increased; therefore, these substrates are more suitable for plants with a short growth cycle. Substrates originate from minerals are included in inorganic materials and thus these substrates are

suitable for both plants with a short growth cycle and plants with a long growth cycle. [9, p. 84–87.]

5 Ethereal Oils

Ethereal oils are aromatic and highly volatile organic chemical compounds [10, p. 3]. Most of the ethereal oils are slightly yellow or completely colourless. The odour is affected by various factors such as species or origins of plants. [11.]

Ethereal oils are part of the secondary metabolites of plants. Ethereal oils are classified especially in two groups, which are terpenes and phenylpropanoids. Ethereal oils are biosynthesized via three different metabolic pathways: mevalonate pathway, non-mevalonate pathway and shikimic acid pathway. [11.]

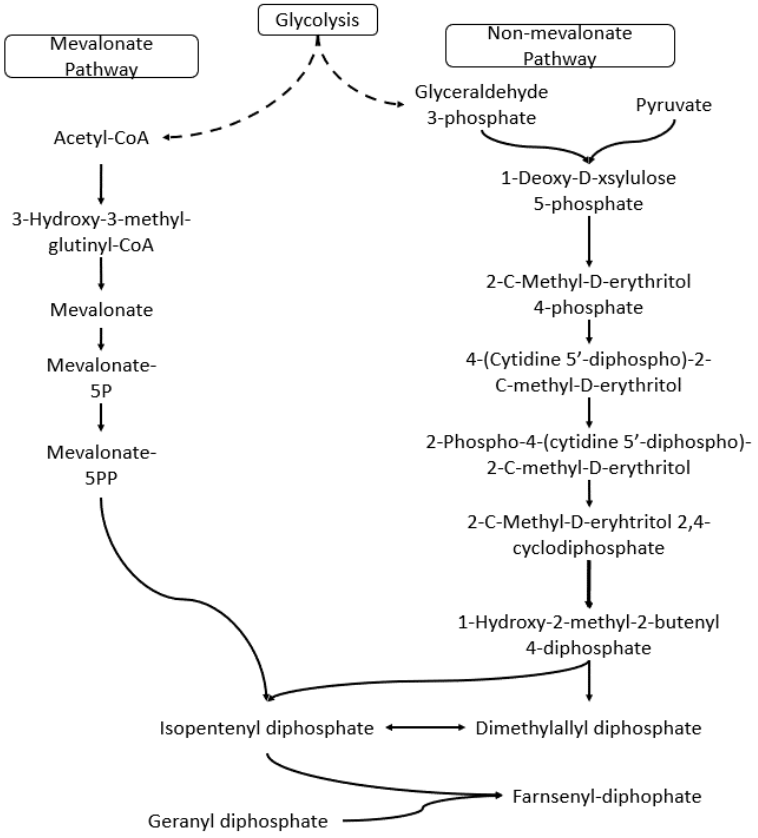

Figure 2. Biochemical flow diagrams of mevalonate and non-mevalonate pathways [12.] Adapted from "Identifying essential genes in bacterial metabolic networks with machine learning methods", by Kitiporn Plaimas; Roland Eils & Rainer König, 2010, BMC Systems Biology, 4, p. 10. CC BY.

Both mevalonate and non-mevalonate pathways lead to synthesisation of terpenes. The differentiative segment is the way to synthesisation of isopentenyl compound as seen in Figure 2. [13.]

The mevalonate pathway requires the synthetization of mevalonic acid (C_6), which is rearranged to form an isopentenyl phosphate with isoprene branches by means of mevinolin enzyme. These isoprene branches are joined to two phosphate groups. [11, 13.]

In the non-mevalonate pathway, glyceraldehyde phosphates are condensed to isopentenyl pyrophosphate with the help of DXP and MEP enzymes. [11, 13.]

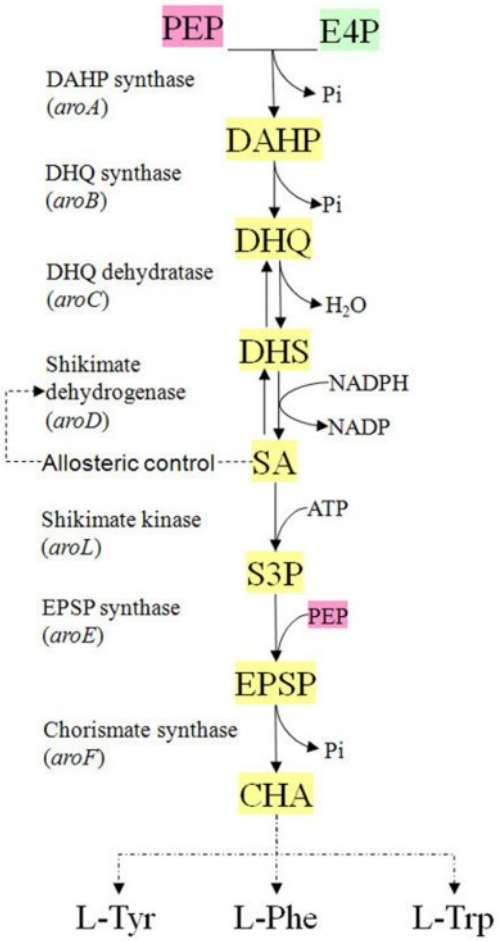

Figure 3. Biochemical flow diagram of shikimic pathway [14.] Adapted from "Metabolic flux responses to genetic modification for shikimic acid production by Bacillus subtilis strains", by Dong-Feng Liu; Guomin Ai; Qing-Xiang Zheng & C. Liu, 2014, Microbial Cell Factories, 13, p. 2. CC BY.

Shikimic acid pathway leads to synthetisation of phenylpropenes. This pathway is completely different from the mevalonate and non-mevalonate pathways (Figure 3). Shikimic acid pathway requires seven carbon atoms to biosynthesise shikimic acid. These seven carbon atoms are obtained from carbohydrates. Shikimic acid transform to chorismic acid via 1,2- and 1,4-eleminaitions of phosphate. The pathway continues with PLP-mediated transamination of chorismic acids to phenylalanine. Phenylalanine ammonia lyase eliminates NH_3 from the phenylalanine, which transforms to cinnamic acid. Through various enzymatic processes, phenylpropenes are one of the possible compounds to be synthesised. [15.]

Ethereal oils are utilized by humans and plants differently. Ethereal oils are attractors for pollinators, repellers against pests/predators and part of the immune system for plants [16, 17]. However, aside from the antibacterial and pest repelling uses of ethereal oils, ethereal oils are applied in cosmetics (e.g. perfume, skincare products and soaps), researched for pharmacological uses (e.g. against ischemic heart disease and antidiabetic effects) and benefitted in food technology (mainly under food safety and flavour) [18].

The end results of the ethereal oil composition of basils are influenced by their environments. For instance, Fiji originated basil samples contain 22.3% linalool and 24.7% methyl eugenol out of total ethereal oil composition, which is about 0.2% of basil or Cuban samples contain 5.4% eucalyptol and 5.0% linalool and ethereal oil content is around 1.9–2.5%. [19.] Linalool, eugenol and eucalyptol are the dominant ethereal oils of European basil, whereas high methyl eugenol content is displeasing. Basils with high linalool or eugenol levels are considered to be European type and high quality. Flavour of basils are affected by ethereal oils composition and alteration of ethereal oils separately is possible only when the metabolic pathways are different. [20.]

6 Experimental Methods

8 basil plants of the same species, *Ocimum basilicum* L., were cultivated as parallel samples. Throughout the project; cultivation was observed, analyses were executed and the physical properties of basil plants were measured.

6.1 Equipment and Materials

Equipment and materials used in this project are listed in Table 1 with their manufacturers and models.

Table 1. Manufacturers and models of equipment and materials

Equipment	**Manufacturer**	**Model**
Greenhouse	-	-
Hydroponic system	Atami	Wilma Mini 8
Led tubes	Valoya	L28/AP67
Humidifier	Senz	SEHU06011
Temperature/humidity sensor	Adafruit	DHT22
Microcontroller	Particle	Photon
Light meter	Delta Ohm	HD 2302.0
pH/EC meter	Mettler Toledo	SevenGo Duo pro
N/P test kits	Hach lange	LCK 339/349
High temperature thermostat	Hach lange	LT 200
Spectrometer	Hach lange	DR 3900
Distillation apparatus	Ace Glass Inc.	Hickman-Hinkle
HS-GC-MS	Shimadzu	HS-20
EDX	Shimadzu	7000
Materials		
LECA	Gold Label	-
Perlite	Plant!t	-
Cellulose fibre		-
Textile fibre	Dafecor Oy	Finntex-suojamatto
Water-soluble fertilizers	Kasviportaat Oy	Supragrow/SupraNitro

6.2 Cultivation Plan

Basils were cultivated in a custom-build greenhouse, which is located in a container in UrbanFarmLab of Metropolia (Figure 4).

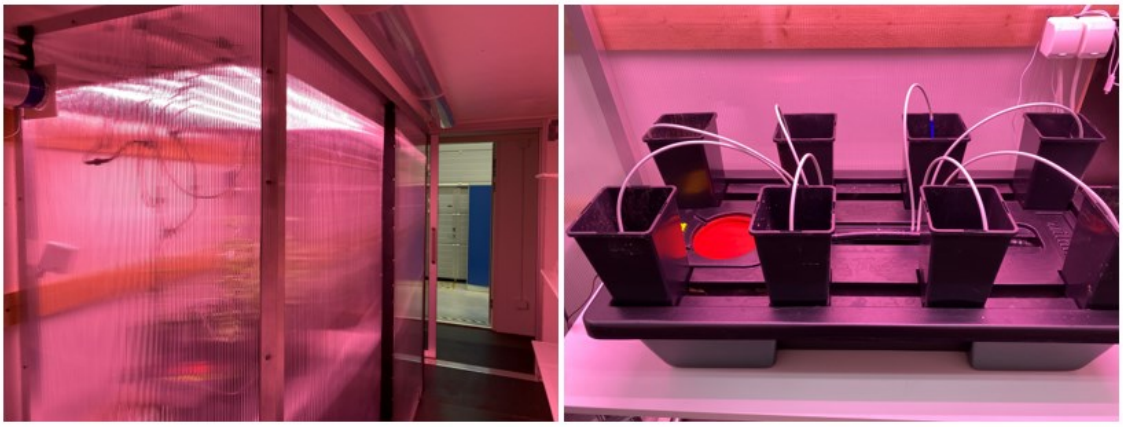

Figure 4. Greenhouse inside the container on the left side and the Wilma Mini hydroponic system on the right side

Hydroponic system only supports up to 8 pots to be placed as shown in Figure 4. The water tank of the system can hold up to 50 L of water/nutrient solution. In-built irrigation system was set to work thrice a day for 15 minutes each time.

The temperature inside the greenhouse is aimed to be around 24 °C and relative humidity around 70%. Humidifier runs every hour for 15 minutes and holds up to 3.5 L of water, which is enough only for about two and a half days and requires adding fresh water to the water tank. Led tubes (AP67) are hanged about 80 cm above the surface of the pots topped with substrate and luminous intensity is around 120 µmol/m2s. The led tubes are on for 21 hours per day from 12 AM to 3 AM. According to Valoya Oy, the manufacturer of led tubes, the spectrum of the led tubes used in this project was; ultraviolet 0%, blue 12%, green 16%, red 57% and 16% [21].

The water tank of the hydroponic system is filled up to 30 L of water and 120 ml of each nutrient solutions, SupraGrow (10%) and SupraNitro (10%), was added. Company's guide on choosing the desired nutrient solutions is followed (see

Appendix 1) and modified according to pervious basil cultivation experiments preformed at UrbanFarmLab.

6.2.1 Preparation of the Cultivation Pots

Each pot of the hydroponic system can hold up to 2.3 L of substrate. The pots were nearly filled to the maximum capacity (Figure 5). LECA and perlite were rinsed to remove the dust before filling the pots. Cellulose and textile fibres were lightly watered to moisten, considering that water disperses more slowly in these materials.

Figure 5. Examples of pots filled with substrates

To each cultivation pot a basil cutting was planted. Basil cuttings were prepared from commercial basils that had grown for around three weeks. Each cutting was about five centimetres long and were rinsed with water before they were planted.

6.2.2 Planting plan

As in Figure 6; the 2 pots on the farthest left contain LECA, the 2 pots on the middle left contain cellulose fibres, the 2 pots on the middle right contain textile fibres and the last 2 pots on the farthest right contain perlite.

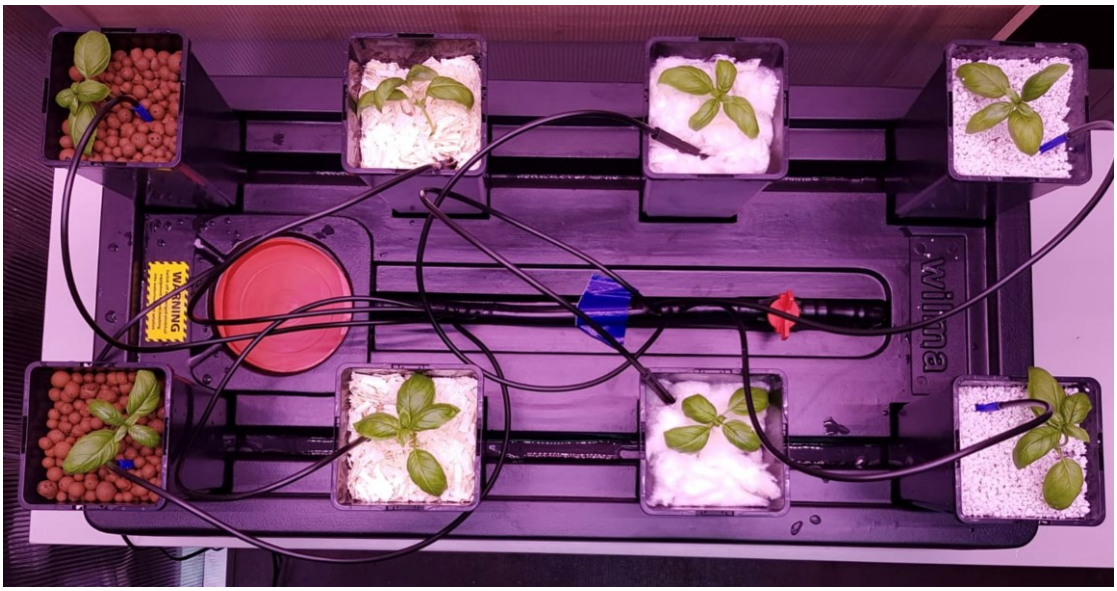

Figure 6. Top-down photo of hydroponic system

Through the project, basils are named as follows: the basils on the upper side of Figure 6 are the first of their parallels (e.g. LECA 1, Perlite 1) and the basils on the lower side are the second (e.g. LECA 2, Perlite 2 etc.).

6.3 Analyses and Measurements

To identify the experiment's performance, several parameters were either measured or analysed during the cultivation periods and at the end of each cultivation period:

- pH and conductivity of substrates
- pH, conductivity and nitrogen and phosphorus concentration of nutrient solution
- temperature and relative humidity of green house
- mass, height, number of leaves and ethereal oils of basils.

6.3.1 pH and Electrical Conductivity of the Nutrient Solution and Substrates

Nutrient solution samples were taken directly from the irrigation pipeline to a 40 ml sample jar, which pH and conductivity was measured from. Before taking the last sample (Week 4), fresh water was added to nutrient solution.

Substrate samples were prepared from dry substrates, which were taken before cultivation period. Dry substrates were mixed with 50 ml of water in a beaker and stirred together for about a minute. Mixture was put on hold for over an hour. Before starting to measure pH and conductivity, mixture was stirred rapidly for a second.

Each sample was measured with the Mettler-Toledo SevenGo Duo Pro device equipped with both a pH and a conductivity electrode. Every week the measuring device was calibrated before the measurements.

pH measurements were performed to ensure that nutrient solution's pH level would not exceed the optimum pH level for basil plants. Putievsky and Galambosi [6] report that basil can be cultivated with a pH level between 4.3–8.2. However, electrical conductivity was measured to determine the consumption of nutrient substances.

For substrates, pH and conductivity measurement was executed once to confirm, that substrate was not too acidic or too basic for basils to grow on these substrates.

6.3.2 Nitrogen and Phosphorus Concentration of the Nutrient Solution

Nitrogen and phosphorus analyses were performed before and after the cultivation periods. Before taking the second sample (Week 4), fresh water was added to nutrient solution. Nitrogen and phosphorus concentrations of the nutrient solution were analysed with the Hach-Lange DR 3900

spectrophotometer. Samples were pre-treated with cuvette test kits according to their manual.

Two layers of disposable nitrile gloves, laboratory coat and safety glasses were worn, and every step is executed under a fume cardboard as a safety precaution, as this method includes usage of strong acids.

6.3.3 Temperature and Relative Humidity of Greenhouse

The main idea behind the temperature and relative humidity measurements was to confirm that greenhouse, humidifier and the ventilation system worked as expected. Both factors were measured with sensors linked to a microcontroller. The microcontroller published the data to Device Cloud. Google Cloud Platform, which was integrated to Device Cloud, received the raw data. Raw data was processed with Cloud Function of Google and was written to Google Sheet file.

The relative humidity of greenhouse was between 60-80% and the temperature was around 24°C.

6.3.4 Height, Mass and the Leaf Quantity of Basils

The heights of basils were measured day-to-day except for the weekends. Leaf counting and weighing were completed at the end of the cultivation period. Before weighing the basil, roots were cut off; however, leaves and the stem remained intact. Measuring was executed with a scale accurate to two decimal places. Leaf counting was carried out after the weighing. Each leaf, from the recently sprouted leaves to completely grown leaves, was counted and picked.

6.3.5 Extraction of Ethereal Oils

Ethereal oil analysis was performed at the end of each cultivation period. Analysis was carried out with the freshest possible basil leaves. Before the analysis, ethereal oils were extracted from basil leaves. The microscale

Hickman-Hinkle distillation apparatus, which includes a 5 ml round-bottom flask, a Hickman head and a water condenser, was used for extraction (Figure 7). In addition, a sand bath and a heat plate were required to heat the sample. The distillation apparatus was assembled under a fume hood for safety measurements.

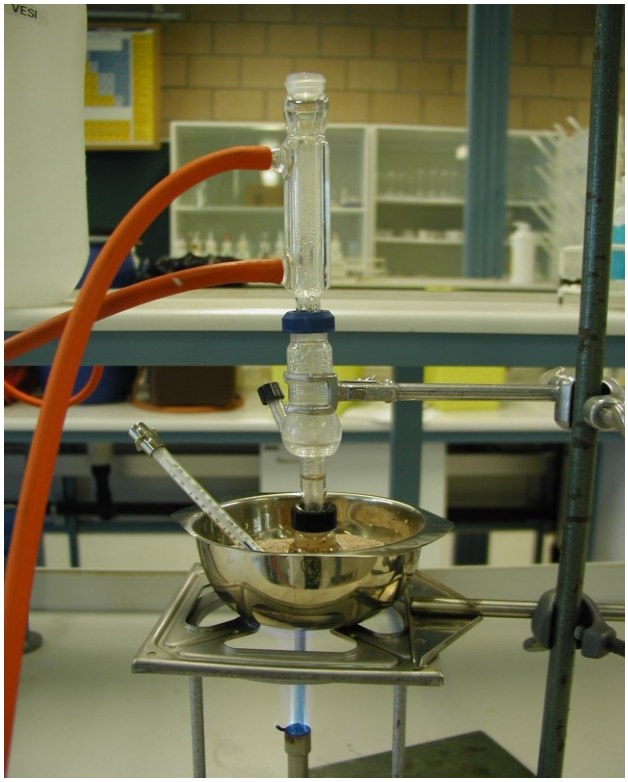

Figure 7. Assembled Hickman-Hinkle distillation apparatus

The microscale distillation apparatus was attached to a standard laboratory stand. The round bottom flask was filled with 1 g leaves and 4 ml of deionized water. The leaves were cut into little pieces with scissors before distilling them, which increases the surface area for ethereal oils to get released and for leaves to fit into the flask. The temperature of the sand bath had to be stabilized around 150–160°C. The distillation process took at least one hour.

6.3.6 Chromatographic Analysis of Ethereal Oils

The distillate, prepared in Chapter 6.3.5, was transferred into a Head-Space vial. The HS-GC-MS analyses were solely qualitative. The vials are inserted into Head-Space sampling system of GC-MS instrument. Parameters of HS-GC-MS analysis are presented in Table 2. AOC-20i+s was used as injector and helium was used carrier gas. The complete parameter table is found in Appendix 2.

Table 2. HS-GC-MS parameters used in this project

HS Conditions		GC Conditions		MS Conditions	
Oven Temperature	70 °C	Injection Temperature	280 °C	Start Time	0 min
Transfer Line Temperature	150 °C	Injection Mode	Split	End Time	27 min
Pressurizing Gas Pressure	100 kPa	Pressure	50.5 kPa	Start m/z	45
		GC Cycle Time	40 min	End m/z	400
		Oven Temperature Rate	10 °C/min 40 °C -> 300 °C		

7 Results

Results are presented under two different groups: Observational results and analytical results.

7.1 Observational Results

At the end of the cultivation period, both pots of cellulose fibres and textile fibres were heavily contaminated with mould. The mould contamination was first found on the cellulose fibre in the first days of cultivation period. The mould contamination on the cellulose fibre was visible mainly on the surface area of the pots and around the roots of basils as can be seen in Figure 6. The mould contamination on the textile fibre was only visible around the emitters of the

irrigation system; spread around the roots and the surface of the pots were clear (Figure 8). The mould contamination developed to the extent of mushroom pilei growth as seen in Figure 6. Each basil growing on textile fibre and cellulose fibre had visible necrosis on the bottommost leaves, Figure 6. Roots of basils grown on cellulose fibre and textile fibre were extremely thin, necrotic and intermingled with the substrates. The mould contamination was spread to the nutrient solution.

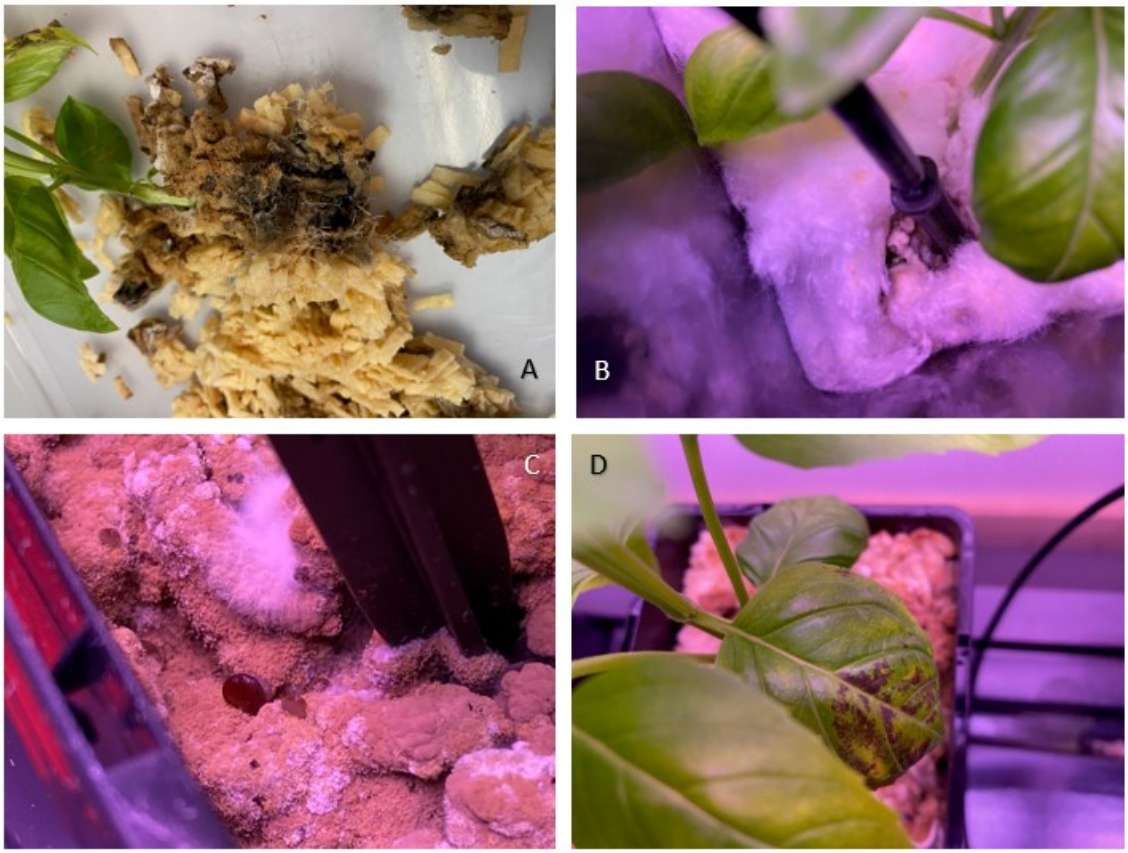

Figure 8. A is a picture of mould surrounding basil roots in cellulose fibre, B is a picture of mould growing in around the emitter, C is a picture of a mushroom pileus sprouted on cellulose fibre

Pots of LECA and perlite were visibly not contaminated with mould; however, both pots of perlite were infested with green algae on the surface. LECA were completely removed for roots, in contrast where perlite remain among roots (Figure 9). Furthermore, each root of basils growing on LECA and perlite were slightly brown. Under the stereomicroscope, part of roots looked as if they were

withered (Figure 9). Leaves of the basils grown on LECA and perlite had slight chlorosis.

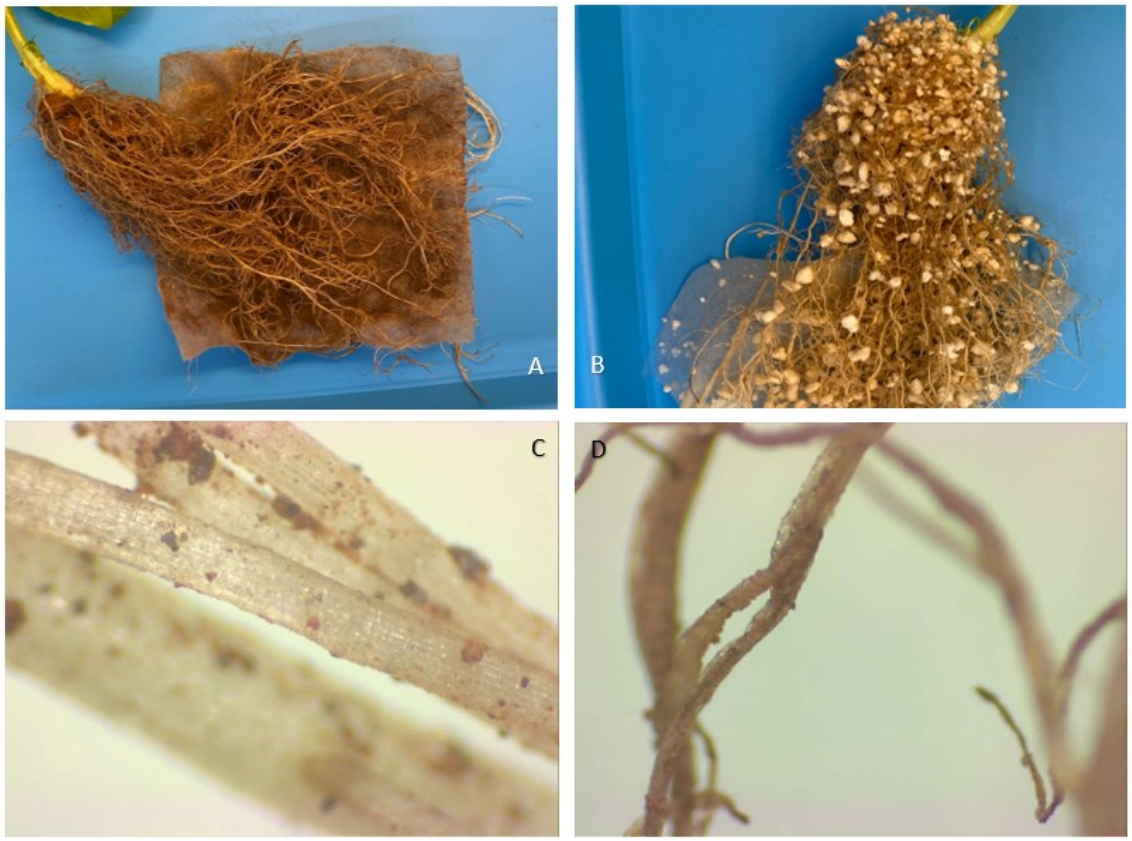

Figure 9. A is a picture of basil root grown in LECA, B is a picture of basil root grown in perlite, C is a picture of LECA healthy basil root grown in LECA under stereo microscope and D is a picture of withered basil root grown in LECA under stereo microscope

The complete daily observation logbook can be found in Appendix 3.

7.2 Analytical Results

Analytical results consist of each result from the laboratory analyses and measurements of basil crops.

7.2.1 Nutrient Solution Analysis Results

As shown in Figure 10, electric conductivity of nutrient solution was 1115 µS/cm at the beginning of the cultivation period. On the first week electric conductivity rose to 1184 µS/cm and drops to 648 µS/cm towards the end of the cultivation period.

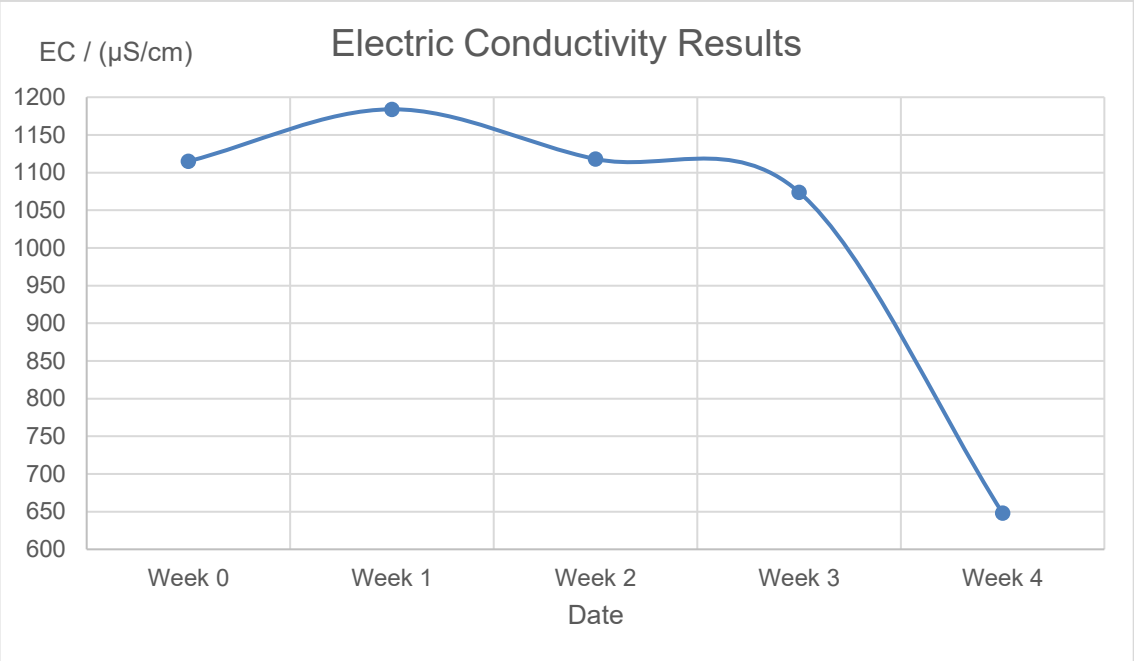

Figure 10. Weekly conductivity results of nutrient solutions

As shown in Figure 11, pH level of nutrient solution was 6.03 at the beginning of the cultivation period. pH level was at its peak on the third week with 6.93. On the last week, pH level dropped to 6,67.

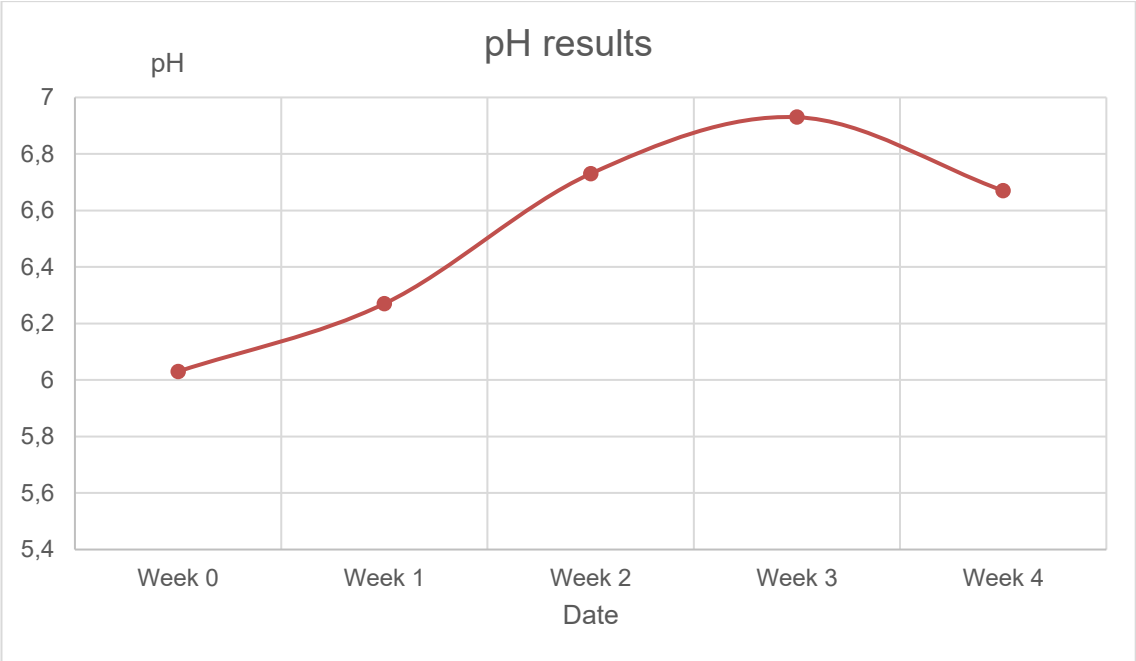

Figure 11. Weekly pH results of nutrient solutions

Nitrogen and phosphorus levels of nutrient solution were only measured twice during this project, thus there are not graphics. The cultivation period started with 487 mg/L of nitrogen and 20.8 mg/L of phosphorus. At the end of the cultivation period, phosphorus was consumed to 2.7 mg/L and nitrogen to 63.4 mg/L.

7.2.2 pH and Electric Conductivity Results of Substrates

The results of pH and electrical conductivity measurements of each substrate are presented in Table 5. The measurement method explained in Chapter 6.3.1 was followed.

Table 3. pH and electrical conductivity results of substrates

	pH	Electrical Conductivity (µS/cm)
LECA	9,22	799
Perlite	10,19	227
Textile Fibre	5,56	818
Cellulose Fibre	8,86	38,3

According to the result of measurement device; LECA, perlite and cellulose fibre are alkaline, with perlite being the most alkaline and textile fibre is acidic with a pH of 5.56. As for the electrical conductivity, textile fibre is the most electrical conductive material with 818 µS/cm and cellulose fibre is the least electrical conductive material with 38.3 µS/cm.

7.2.3 Ethereal Oils Analysis Results

As shown in Table 6, only the four of the ethereal oils, from the complete report of GC-MS were gathered as those were the priority of this project (see Appendix 4 for the complete report).

Table 4. Results of the HS-GC-MS of the basils

Substrate	Eucalyptol (%)	Linalool (%)	Eugenol (%)	Methyl eugenol (%)
LECA 1	19.65	31.41	15.25	4.7
LECA 2	26.09	30.44	21.95	0.94
Perlite 1	7.95	44.42	19.1	-
Perlite 2	18.16	54.21	16.78	-
Textile Fibre 1	23.08	29.1	22.93	8.71
Textile Fibre 2	26.81	33.93	11.03	10.75
Cellulose Fibre 1	21.51	41.36	27.43	-
Cellulose Fibre 2	20.29	36.5	23.11	5.34

Linalool is the dominant ethereal oil of basils according to Table 6. The linalool level of basil grown on Textile Fibre 1, 29 %, is the lowest compared to other

basils, while the basils grown on perlite are the richest in linalool with 44 % and 54 %. The levels of eucalyptol are around 27–18 % with the exception of Perlite 1, which have 8 % eucalyptol and the level of eugenol is varied between 11–27 %. Methyl eugenol levels are quite low compared to eucalyptol, linalool and eugenol. Methyl eugenol is completely missing in basils grown on perlite and Cellulose Fibre 1. Methyl eugenol is detected up to 11 %.

7.2.4 Quantitative Measurement Results of Basil Crops

In Figure 12, day-to-day height measurement of each basil plant is presented, and the basil plants grown on the same type of substrate have the same line and marker colour.

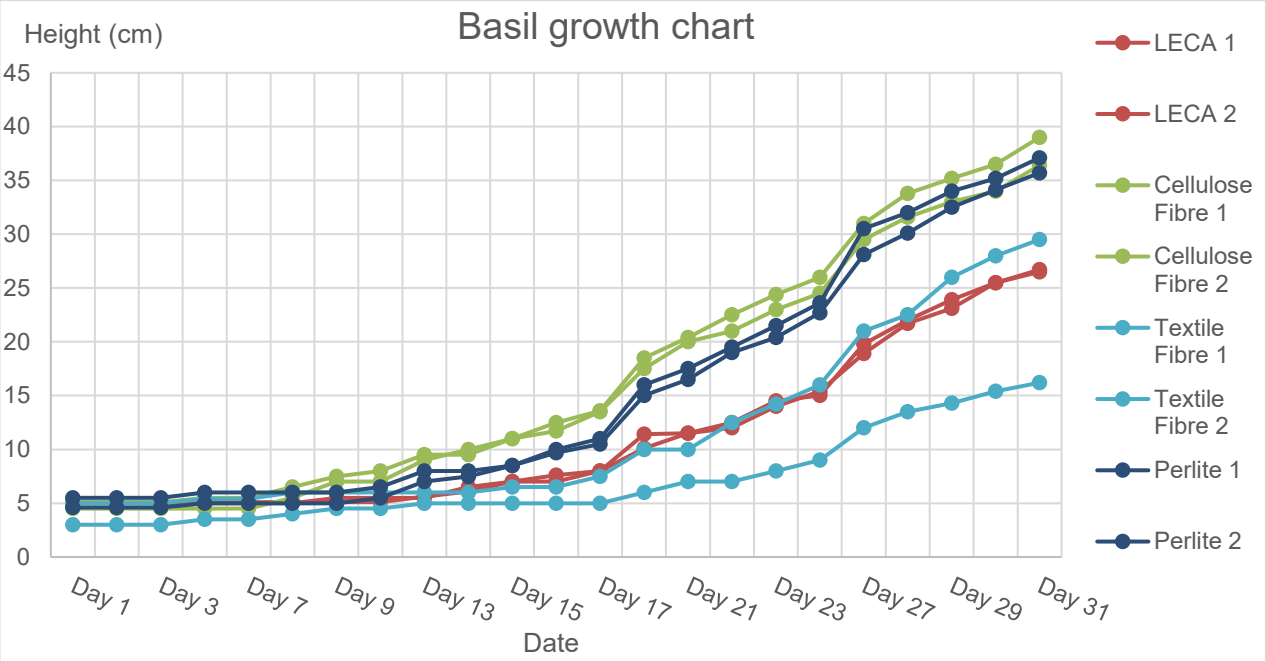

Figure 12. Daily measurements of basil height

Each basil cutting was about 5 cm long at the beginning of the cultivation period, besides the basil grown on Textile Fibre 1, which was 3 cm long. The basils were growing on rooting stage and the average growth on this stage was around 1.5 cm. The fastest transition from rooting to steady growth was basils planted on Cellulose Fibre 2, on the day 5. Steady growth of basil on Cellulose Fibre 1 started on day 8, basil on LECA 1 and 2 started on days 11 and 9 and

basils on Perlite 1 and 2 started on days 8 and 10. The slowest transition from rooting to steady growth was basils on Textile Fibre 1 and 2, which started on day 13 and 12.

The growth of each basil on perlite and cellulose fibre was superlative compared to basils of LECA and textile fibre. On the twenty-third day, the final heights of basils were 26.5 cm (LECA 1), 26.7 cm (LECA 2), 39.0 cm (Cellulose Fibre 1), 36.4 cm (Cellulose Fibre 2), 16.2 cm (Textile Fibre 1), 29.5 cm (Textile Fibre 2), 37.1 cm (Perlite 1) and 35.7 cm (Perlite 2).

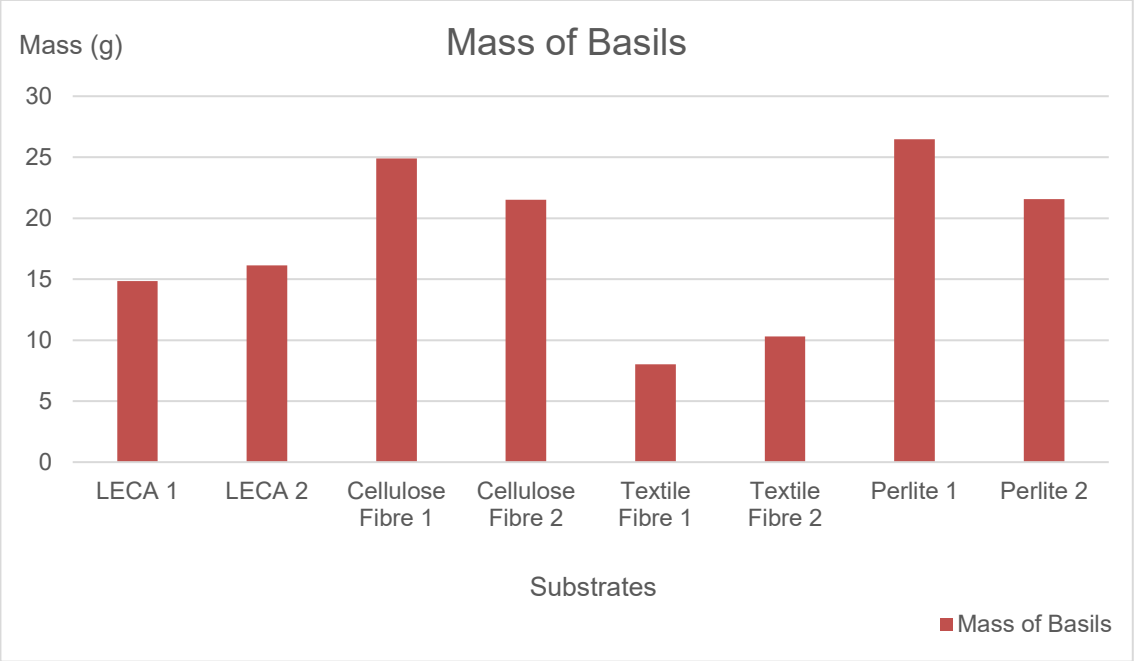

Figure 13. Mass of each basil at the end of cultivation period

In Figure 13, masses of crops of all eight basil plants are presented. At the end of the cultivation period basils growing on textile fibres were the lightest with 8 g and 10 g. Basils growing on Perlite 1 and Cellulose Fibre 1 were the heaviest with 2 g of gap between the two basils. Mass of both basils growing on LECA were close to the average mass obtained in this project.

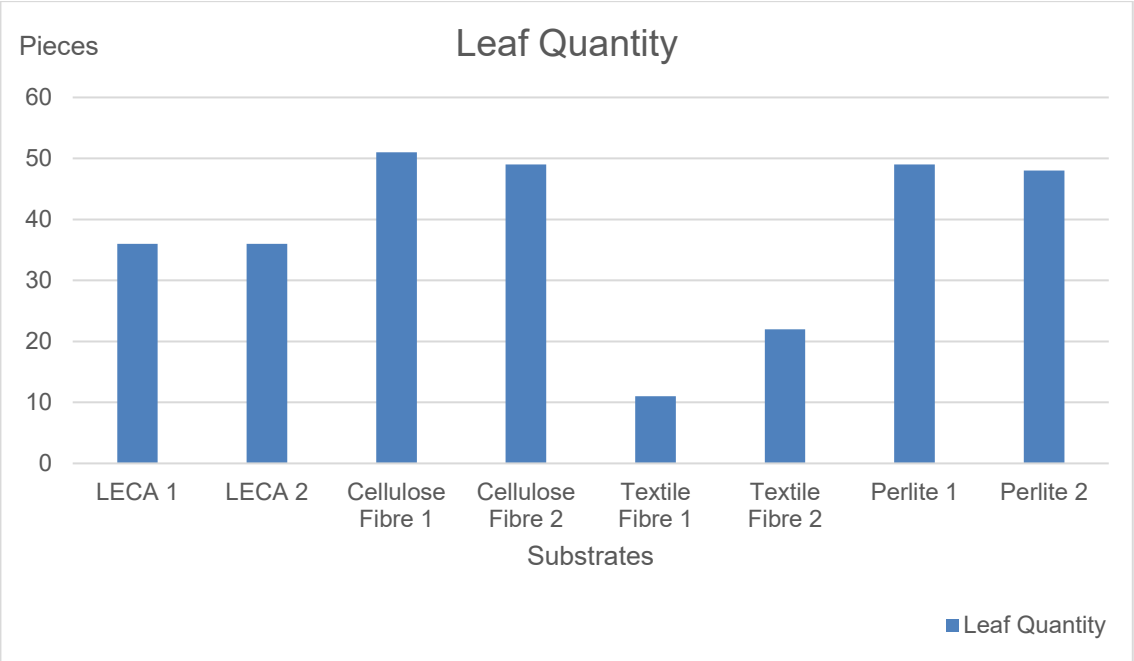

Figure 14. Leaf quantity of each basil at the end of cultivation period

All in all, the basils growing on textile fibres had the least leaves with 11 and 22 pieces as seen in Figure 14. Basils on perlite and cellulose fibre had the most leaves between the 51–48 pieces. Both basils growing on LECA had leaves around the average quantity obtained in this project.

8 Discussion

The main point of this project was to examine various substrates to determine whether they had any effect on basils growth and ethereal oil composition. The pH analysis results of the substrate indicates that these substrates are suitable for basil to cultivate on, except for perlite which had a higher pH level than the maximum optimal pH level for basils. However, perlite is used in hydroponic systems in general and was overlooked in this project. Furthermore, the dynamic pH level of the nutrient solution prepared for this project was optimal for basil throughout the cultivation period.

Nutrients in the nutrient solution were consumed roughly completely. This is supported by the decrease of electric conductivity level and nitrogen and

phosphorus concentration in nutrient solution as can be seen from data of Chapter 7.2.1. Although it is not known whether cellulose fibres and textile fibres caused an error on the results by absorbing the nutrients away from the plant and irrigation cycle. Nevertheless, basils developed well which is another evidence of nutrient being consumed.

Basils grown on LECA having the average height, mass and leaf quantity could be caused by the properties of the LECA. LECA did not hold water adequately and dried rapidly, the irrigation schedule may have been insufficient for basil growing on LECA. In addition, water flows through LECA greater than through other substrates used in this project. Greater water flow leads to less nutrient intake if the irrigation schedule is not sufficient. Thereby basils grown on LECA were inferior to ones grown on cellulose fibre and perlite.

Basils of cellulose fibre had one of the best results in harvest wise; height, mass and leaf quantity, yet (as it was foreseen beforehand) mould contamination was a serious problem. Cellulose fibres are estimated to be the starting point of mould contamination in the whole system, since the mould was first observed on cellulose fibre. As cellulose is formed from series of glucose and was regularly watered, it was a perfect medium for mould to grow. It is unknown whether cellulose fibre was already contaminated, or mould spores were in laboratory air or mould was accidently carried by someone. The latter option is not possible, due to aseptic working techniques.

Mould contamination on cellulose fibre may explain the fast growth of basils on cellulose fibre. The enzymes of mould break cellulose to glucose segments and mould uses glucose in the metabolic process. Output molecules of these processes may become additional supplement to basils depending on the species of mould that contaminated the cellulose fibre. However, the relation between the mould and the basils are not known and only estimated to be a parasitic rather than mutualistic. The last statement is supported by the necrotic leaves found on the lower levels of the basils of cellulose fibre and textile fibre.

Also roots of the same basils being withered, thin and surrounded by mould suggests the parasitic relation.

Basils grown on perlite had the second-best results alongside the ones grown on cellulose fibre; nonetheless, the growth rate of basils grown on perlite was slower and overall results are lower. This can be explained with the hypothesis presented in the previous paragraph. Like with LECA, water flows through perlite without a great resistance, yet perlite stayed moist for longer periods. Therefore, basils grown on perlite may had been accessing more nutrients than the ones grown on LECA.

The moistness of perlite and the long periods of lighting enabled the infestation of the green algae on the surface. The green algae were examined under an optical microscope but nothing definite was observed to identify the algae. Green microalgae, visibly, was not harmful to basils.

Basils on the textile fibre had a large height and leaf quantity gap. However, differences of mass are less in comparison to basils on the other substrates. Throughout the project the surface of the textile fibre was partly dry, which may indicate that the irrigation method was not compatible with the textile fibre. This may cause nutrient deficiency and underdevelopment. Nevertheless, if the emitter of Textile Fibre 2 is positioned better than that of Textile Fibre 1, the roots of the basil of Textile Fibre 2 may absorb more nutrient.

The mould contamination problem was also existent on both textile fibres. The same patent of behaviour could be perceived around the roots, but mould was not spreading on the surface. This may be explained by surface not being moist enough for mould to survive. Another reason might be, unlike cellulose, textile fibres are not composed of series of glucose or other carbohydrates and mould was only surrounding roots to consume carbohydrates synthesized by basils.

It is difficult to draw any conclusions from ethereal oil analyses, since the analyses gave approximate results; considering that essential oil extraction was executed on a microscale apparatus as mentioned in Chapter 6.3.5, where only

1 gram of leaf mass from each basil was analysed. There are crucial differences between parallel samples. A suitable example for these differences is that the basil grown on Cellulose Fibre 1 did not biosynthesise methyl eugenol, whereas the basil grown on Cellulose Fibre 2 biosynthesised. However, each basil synthesized the three desired ethereal oils (eucalyptol, linalool and eugenol) and these ethereal oils had the highest area percentages.

9 Conclusion

This project was executed to examine the effects of different substrates on the growth of basils and the ethereal oil composition in hydroponic system. Eight basils of same species were cultivated as parallel samples. Each basil was under the same greenhouse conditions and had the same irrigation system.

There were three types of data collected. The observations were collected as written documents, laboratory analyses results were gathered in charts and sensor measurements were uploaded to a cloud service. While sensor measurements were only collected to control the greenhouse conditions, observations and laboratory analyses results were examined to determine an answer to the research questions of this project.

The result of the laboratory analyses indicate that substrates did not have a significant effect on ethereal oil biosynthetisation; nevertheless, effects on mass, leaf quantity and height were considerable. According to results, perlite and cellulose fibre gave better results, but observational result indicated that cellulose fibre is not suitable for hydroponics as a substrate. In conclusion, perlite is the sole satisfactory alternative among the four substrates.

It is suggested that similar projects be executed with separate water tanks to prevent the spread of diseases and harmful chemicals secreted from plants. Examination of one type of substrate per cultivation period will provide more reliable results. Autoclavation of substrates can decrease the possibility of

mould contaminations. More suitable ethereal oil analysis method will present a more precise conclusion.

Special thanks to Carola Fortelius-Sarén, Marja-Leena Åkerman and Kaj Lindedahl, without whom this project would not have been executed.

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Nutrient Solution Guide

Table 1. Required water-soluble nutrient for plants on different stages of circle of life [20]

Salads, Artichoke, Basil and other Herbs			
Nutrient mix for	New plants	Grow	Bloom
Made from 10% Mother liquid of:	**ml/10 L**	**ml/10 L**	**ml/10 L**
SupraStart	20	10	0
SupraGrow	0	40	15
SupraNitro	15	65	25
SupraBloom	5	0	15
SupraMgS	5	15	15
Total	45	130	70
Add to new plant water	**10 L**	**10 L**	**10 L**
Total N-P-K (EU)	10 - 9 - 7	11 - 3 - 10	7 - 6 - 12
Total N-P-K (with oxides)	10 - 19 - 9	11 - 6 - 12	7 - 10 - 15
Target pH	6.0 - 6.5	6.0 - 6.5	6.0 - 6.5
Target EC	0.6 - 1.4	0.6 - 1.5	0.6 - 1.6

HS-GC-MS Parameters

[Comment]

===== Analytical Line 1 =====

[AOC-20i+s]

# of Rinses with Pre-solvent: 0

# of Rinses with Solvent(post): 0

# of Rinses with Sample: 4

Plunger Speed (Suction): High

Viscosity Comp. Time: 0.2 sec

Plunger Speed (Injection): High

Syringe Insertion Speed: High

Injection Mode: Normal

Pumping Times: 5

Inj. Port Dwell Time: 0.3 sec

Terminal Air Gap: No

Plunger Washing Speed: High

Washing Volume: 8uL

Syringe Suction Position: 0.0 mm

Syringe Injection Position: 0.0 mm

Solvent Selection: only A

[GC-2010]

Column Oven Temp.: 40.0 °C

Injection Temp.: 280.00 °C

Injection Mode: Split

Flow Control Mode: Linear Velocity

Pressure: 50.5 kPa

Total Flow: 24.3 mL/min

Column Flow: 1.01 mL/min

Linear Velocity: 36.3 cm/sec

Purge Flow: 3.0 mL/min

Split Ratio: 20.0

High Pressure Injection: OFF

Carrier Gas Saver: ON

Carrier Gas Saver Split Ratio :10.0

Carrier Gas Saver Time :1.00 min

Splitter Hold: OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	40.0	0.00
10.00	300.0	1.00

< Ready Check Heat Unit >

Column Oven: Yes

SPL1: Yes

MS: Yes

SPL2: Yes

MS: Yes

< Ready Check Detector (FTD) >

< Ready Check Baseline Drift >

< Ready Check Injection Flow >

SPL1 Carrie: Yes

SPL1 Purge: Yes

SPL2 Carrier: Yes

SPL2 Purge: Yes

< Ready Check APC Flow >

APC1: Yes

< Ready Check Detector APC Flow >

External Wait: No

Equilibrium Time: 1.0 min

[GC Program]

===== Analytical Line 2 =====

[HS-20]

Oven Temp.: 70.0 °C

Sample Line Temp.: 150.0 °C

Transfer Line Temp.: 150.0 °C

Shaking Level: OFF

Multi Injection Count: 1

Pressurizing Gas Pressure: 100.0 kPa

Equilibrating Time.: 15.00 min

Pressurizing Time.: 1.00 min

Pressure Equilib.Time: 0.50 min

Load Time: 0.50 min

Load Equilib. Time: 0.20 min

Injection Time: 0.50 min

Needle Flush Time: 0.00 min

GC Cycle Time: 40.00 min

Check System Ready: ON

Extended System Ready Check Limit: 45 min

Check GC Ready: ON

Extended GC Ready Check Limit :10 min

Analysis Mode: Constant

Needle Check: Yes

Action on Leak Check Error: Continue

Action with No Vial on Tray: Skip

[GC-2010]

Injection Mode: Direct

Flow Control Mode: Pressure

Pressure :25.7 kPa

Column Flow: 0.69 mL/min

Linear Velocity: 30.0 cm/sec

Purge Flow: 3.0 mL/min

< Additional Flow >

APC1 Pressure: 100.0 kPa

[GCMS-QP2010 Ultra]

Ion Source Temp: 200.00 °C

Interface Temp.: 325.00 °C

Solvent Cut Time: 0.00 min

Detector Gain Mode: Relative

Detector Gain: +0.00 kV

Threshold :0

[MS Table]

--Group 1 - Event 1--

Start Time :0.00min

End Time :27.00min

ACQ Mode: Scan

Event Time: 0.15sec

Scan Speed: 2500

Start m/z: 45.00

End m/z: 400.00

Sample Inlet Unit: GC

Inlet Line: Line 2

[MS Program]

Use MS Program: OFF

Observation Logbook

Week 1

After the first day of the cultivation period stems of basil cuttings planted on LECA became unable to support the basils and textile fibres were compressed by the pressure of basils, volume of the textile fibres was decreased. This caused to surface level of the textile fibres to drop by few centimetres. On the fifth day, basil cuttings on LECA, Perlite and textile fibre started to wither as seen in Figure 1.

Figure 1. The basils on LECA (most left column), textile fibre (middle right column) and perlite (most left column) are the withering basils. The basils on cellulose fibre (middle left column) are healthy.

Cellulose fibre was contaminated with mould, Perlite 1 was populated by green algae although it was vaguely visible, and root sprouts of basils took their shapes under five days. On the sixth day, mould kept spreading on the surface of Cellulose Fibre 1 and the first signs of mould contamination on Cellulose Fibre 2 was found. On the seventh day, about quarter of the surface of the Cellulose Fibre 1 was covered with mould, like in Figure 1, thus contaminated cellulose fibre bits were removed to find if it was possible to prevent the spread of mould.

Figure 2. Yellow/brown mould on the cellulose fibre on the lower right corner of the picture

Week 2

On the eighth day, signs of mould contamination on Cellulose Fibre 2 was found again. On the nineth day, about half of the surfaces of both pots of cellulose fibres were covered with brown and white mould like in Figure 3 and mould with same aspects was found on the surface of textile fibre 4, as shown in Figure 3.

Figure 3. Cellulose Fibre 1 (image on the left) and 2 (image on the right) with mould on the surface

Figure 4. The mould found on the surface of Textile Fibre 1

Between the days 10 and 12, all basils recovered back to healthy state, yet on the bottom leaves of Textile Fibre 1 and Perlite 1 necrosis occurred, in addition the mould on both pots of textile fibre spread around the drip emitters and green algae, that populated the perlite, was more evident (Figure 5). On the twelfth day, although basils recovered from withering, leaves of basils planted on textile fibre were slightly paler than other basils. Besides, mould was noticed on the drainage channel under the pots of cellulose fibres and textile fibres.

Figure 5. Each basils image from the day 12, from left to right substrates are as follows; LECA, cellulose fibre, textile fibre and perlite

On the thirteenth day, surfaces of both pots of cellulose fibre were completely covered with mould, which prevented water from being absorbed like in Figure 6.

Figure 6. Cellulose fibres where mould prevents water from being absorbed by the substrate

Week 3

By the fourteenth day, textile fibres shrank to half of their starting volume. Through the days between 15 to 18, no changes were detected. On the day 19, mushroom pilei are found on both pots of cellulose fibres as in Figure 7.

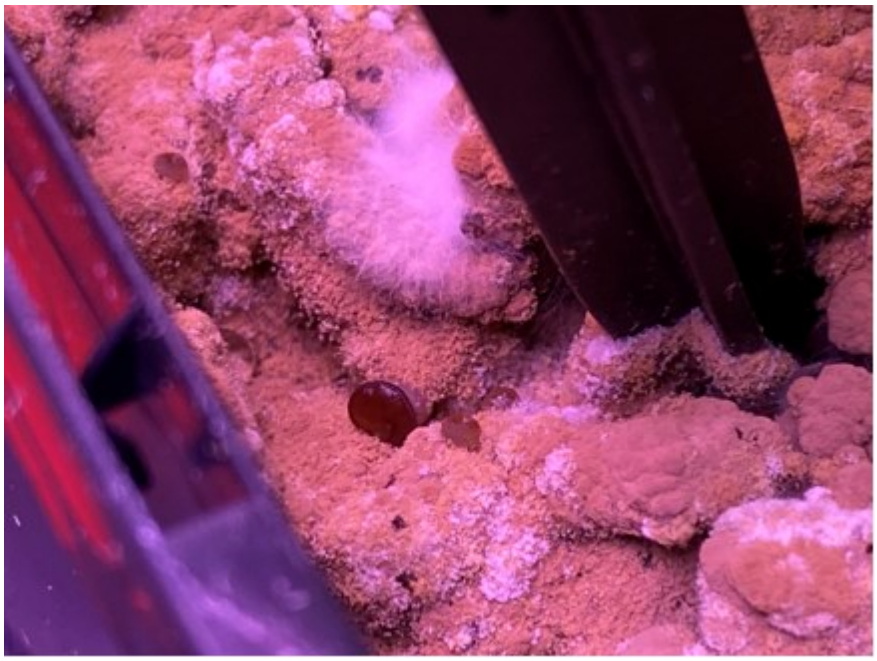


Figure 7. Mushroom pileus on cellulose fibre

By the twentieth day, majority of the surfaces of textile fibres was covered with mould and mushroom pilei are grown. On the twentieth day, mould was found growing on the surface of nutrient solution as seen in Figure 8.

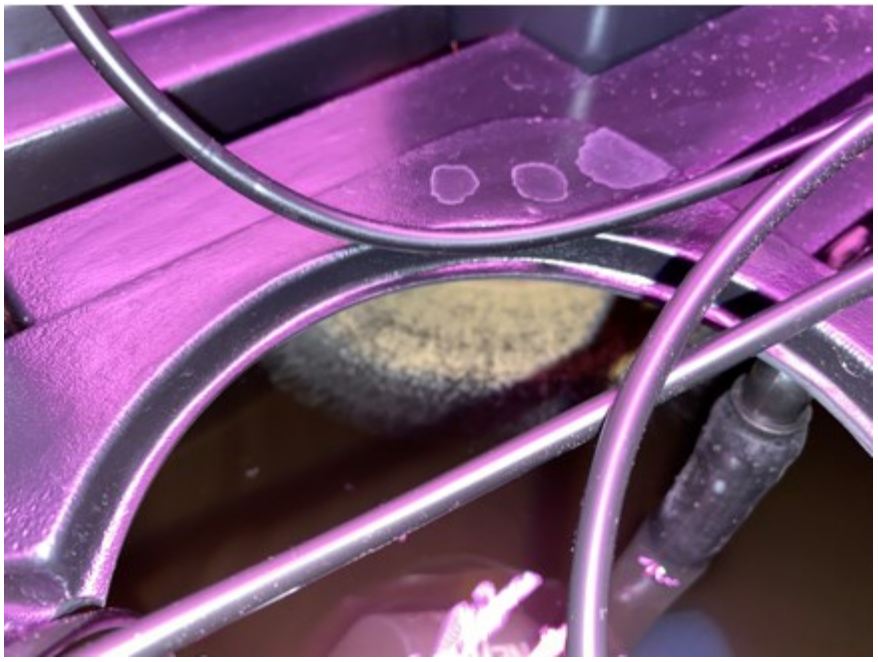


Figure 8. Mould on the surface of nutrient solution

Week 4

On the day twenty-two, necrosis on few leaves of basils growing on cellulose fibre and textile fibre were noticed. Throughout the rest of the cultivation period, only noticeable differences were the spread of the green algae on perlite and spread of the mould on cellulose fibres and textile fibres.

Leaves, Substrates and Roots

Figure 9. Leaves, roots and LECA at the end of the cultivation period

Both roots of basils, that were planted on LECA, were grown out of the drainage holes on the bottom of the pots by piercing through the filter (Figure 9). The roots before the filter were brown, thin and shrunken; however, roots beyond the filter were white/tan and succulent. Observing the roots under a stereomicroscope supported the previous statement and tiny LECA particles

sticking to roots were noticed as seen in Figure 9. There was no visible contamination (mould, green algae…) nor deformation (Figure 9). Both roots of the basils on LECA and both pots of LECA were identical and impossible to differentiate. Both basils planted on LECA had healthy, green leaves but few of the leaves had slight necrosis around the tip and edges like in Figure 9.

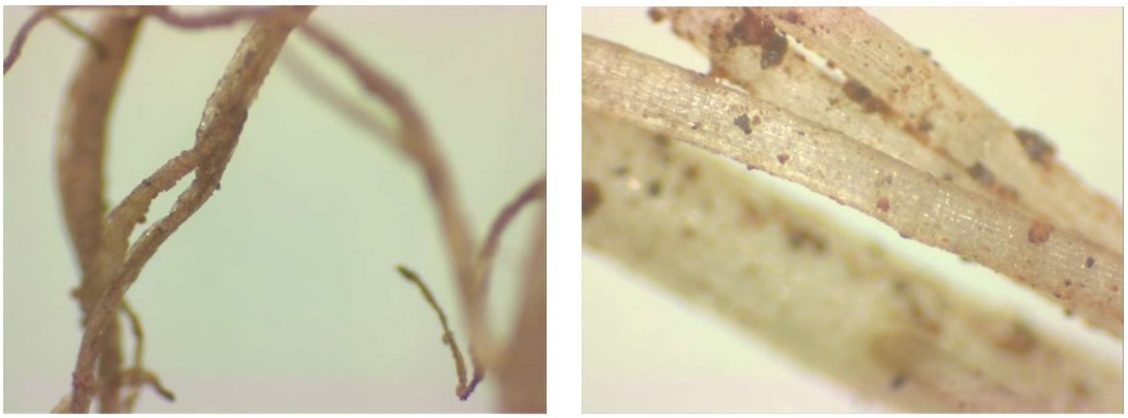


Figure 10. Roots under a stereomicroscope

Roots, that grew in perlite, were grown out of the drainage holes on the bottom of the pots by piercing through the filter. Majority of the roots before the filter was hollow or had signs of necrosis (Figures 11). The roots were mostly covered with perlite. The roots, that could pierce through the filter, were sparse and rather necrotic. Examination under a stereomicroscope supported this observation. Except for the surfaces of both pots of perlite, green algae were not spread to the deeper parts of the pots. Both roots of the basils on perlite and both pots of perlite were identical and impossible to differentiate. Both basils planted on perlite had healthy, green leaves but few of the leaves had slight necrosis around the tip and edges like in Figure 11.

Figure 11. Leaves, roots and perlite at the end of the cultivation period

The cellulose fibres around the roots were completely contaminated with mould like in Figure 12. Mould was leaking through the drainage holes from both pots of cellulose fibres. The majority of the roots were either hollow or withered. Under a stereomicroscope, mould growing out of roots was observed (Figure 12). Both roots of the basils growing on cellulose fibres and both pots of cellulose fibres were identical and impossible to differentiate. Except for the healthy leaves, few of the leaves were terribly necrotic and chlorotic.

Figure 12. Contaminated cellulose fibres, leaves of basil and a stereomicroscopic image of the basil roots

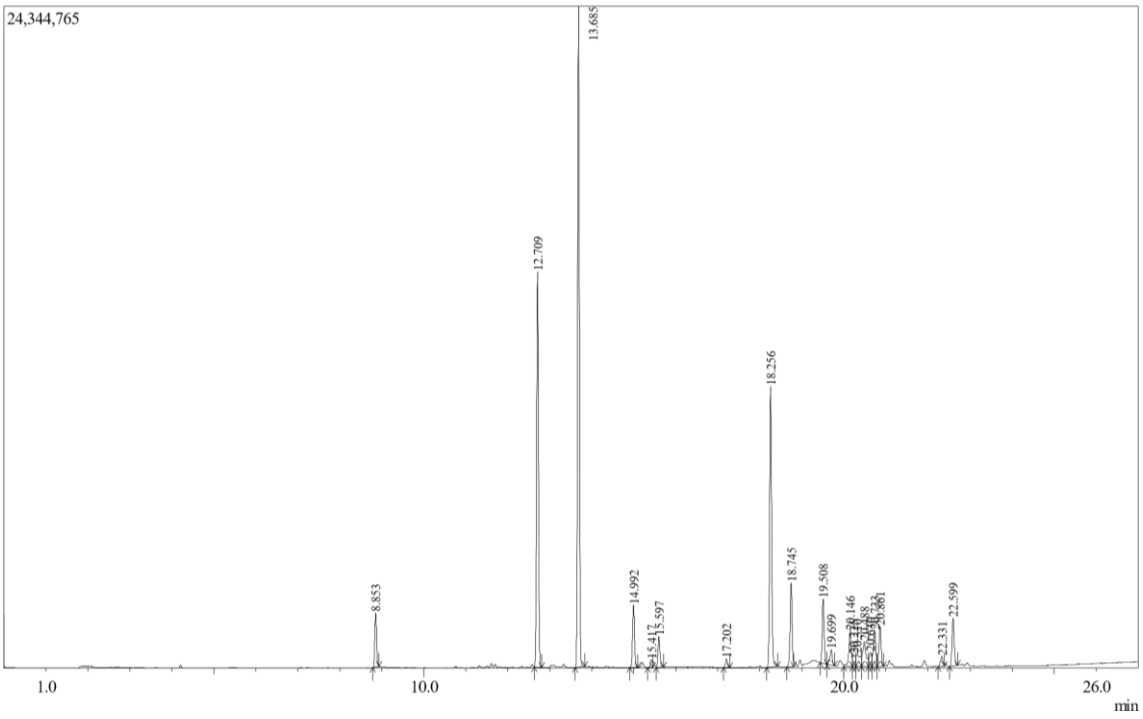
The textile fibres were contaminated with both brown and green mould (Figure 13). The roots of basils that were growing on textile fibre, were nearly impossible to see. The roots were extremely thin and intermingled with textile. Necrotic roots were detected in between the textile fibres, while the roots that were not surrounded by textile, were in a better condition. Mould was growing out of the drainage holes from both pots of textile fibres. Both roots of the basils growing on textile fibres and both pots of textile fibres were identical and impossible to differentiate from each other. Leaves of basils grown on textile

fibres appeared to be healthy; nonetheless, there were slight necrosis on the edges and one leaf was covered with necrosis like in Figure 13.

Figure 13. Basil on textile with rotten roots and leaves of basil

HS-GC-MS Analyses Reports

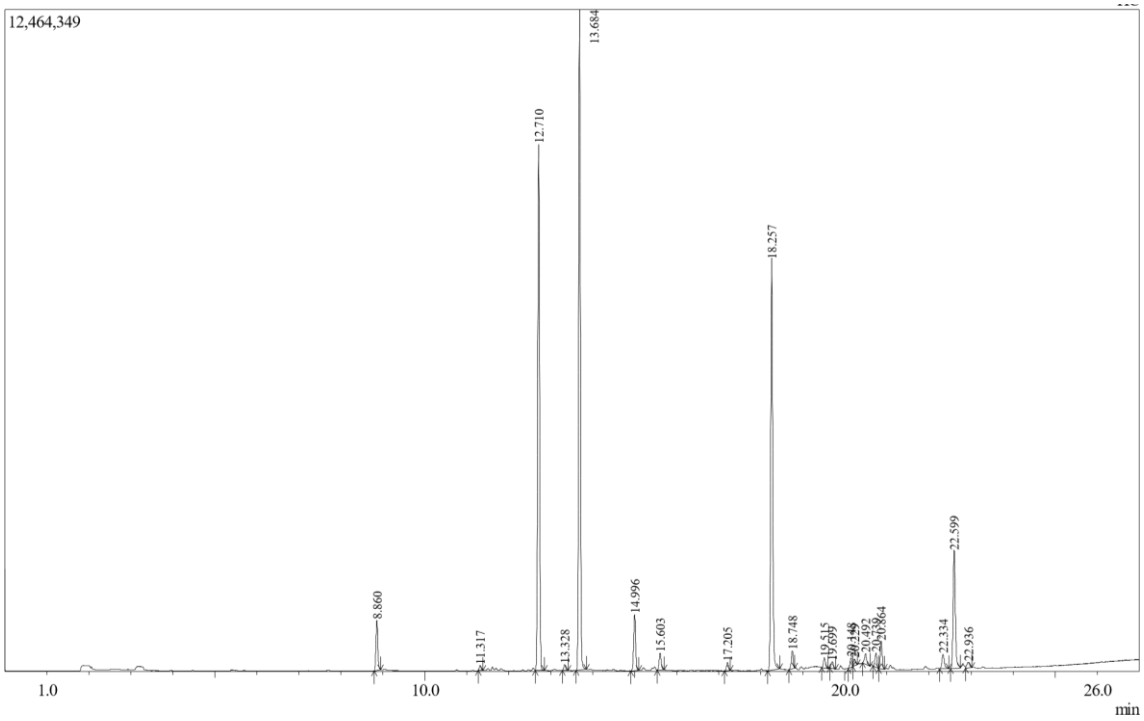
LECA 1 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.853	6029132	2.71	3-Hexen-1-ol, (Z)-
2	12.709	43713746	19.65	Eucalyptol
3	13.685	69885516	31.41	1,6-Octadien-3-ol, 3,7-dimethyl-
4	14.992	7435739	3.34	(+)-2-Bornanone
5	15.417	853922	0.38	Terpinen-4-ol
6	15.597	3655309	1.64	.alpha.-Terpineol
7	17.202	1003813	0.45	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2
8	18.256	33929076	15.25	Eugenol
9	18.745	10485212	4.71	Methyleugenol
10	19.508	7542572	3.39	cis-.beta.-Farnesene
11	19.699	2298381	1.03	.alpha.-Guaiene
12	20.146	5023008	2.26	Humulene
13	20.225	1899461	0.85	(+)-epi-Bicyclosesquiphellandrene
14	20.310	3128864	1.41	
15	20.488	5090254	2.29	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1
16	20.630	2078802	0.93	Naphthalene, decahydro-4a-methyl-1-methylene
17	20.733	5349462	2.40	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
18	20.861	5263027	2.37	.gamma.-Muurolene
19	22.331	1475670	0.66	cubedol
20	22.599	6319098	2.84	.tau.-Cadinol
		222460064	100.00	

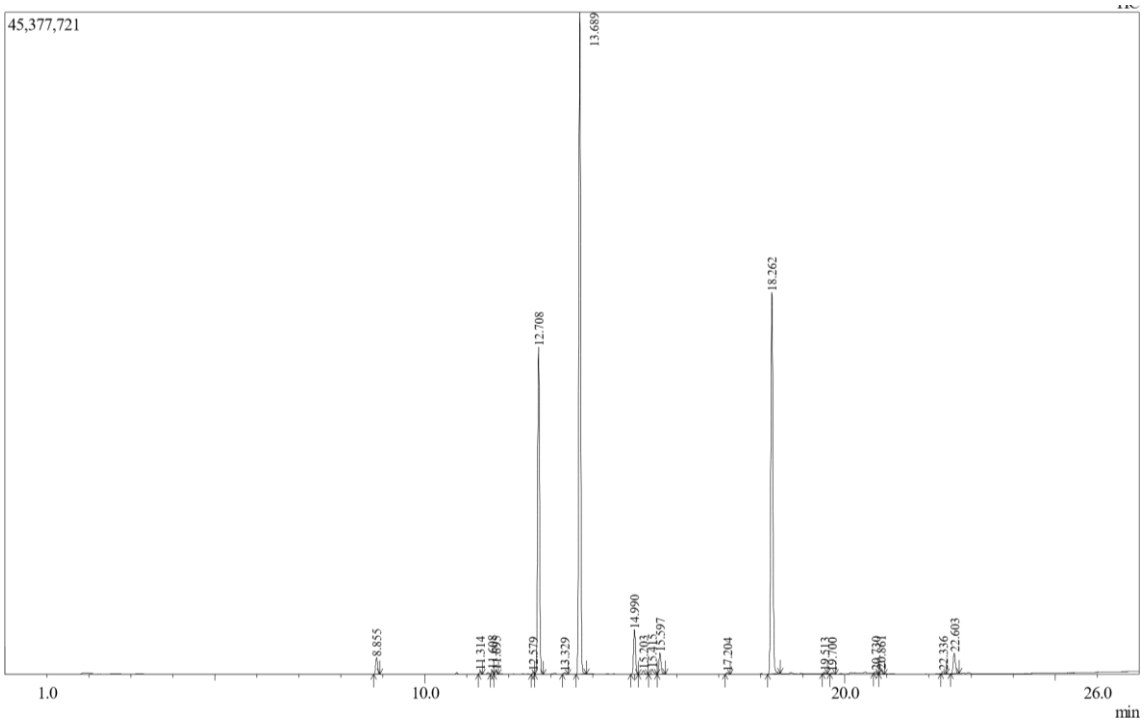
LECA 2 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.860	2849840	2.48	3-Hexen-1-ol, (Z)-
2	11.317	260570	0.23	1-Octen-3-ol
3	12.710	29982349	26.09	Eucalyptol
4	13.328	350089	0.30	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl
5	13.684	34979841	30.44	1,6-Octadien-3-ol, 3,7-dimethyl-
6	14.996	3378421	2.94	(+)-2-Bornanone
7	15.603	1091137	0.95	.alpha.-Terpineol
8	17.205	494435	0.43	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2
9	18.257	25219269	21.95	Eugenol
10	18.748	1083197	0.94	Methyleugenol
11	19.515	658466	0.57	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-me
12	19.699	423457	0.37	.alpha.-Guaiene
13	20.148	561524	0.49	Humulene
14	20.229	424134	0.37	(+)-epi-Bicyclosesquiphellandrene
15	20.492	720093	0.63	.beta.-copaene
16	20.739	859341	0.75	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
17	20.864	1680821	1.46	.gamma.-Muurolene
18	22.334	1082913	0.94	cubedol
19	22.599	8355884	7.27	.tau.-Cadinol
20	22.936	463795	0.40	Cubenol
		114919576	100.00	

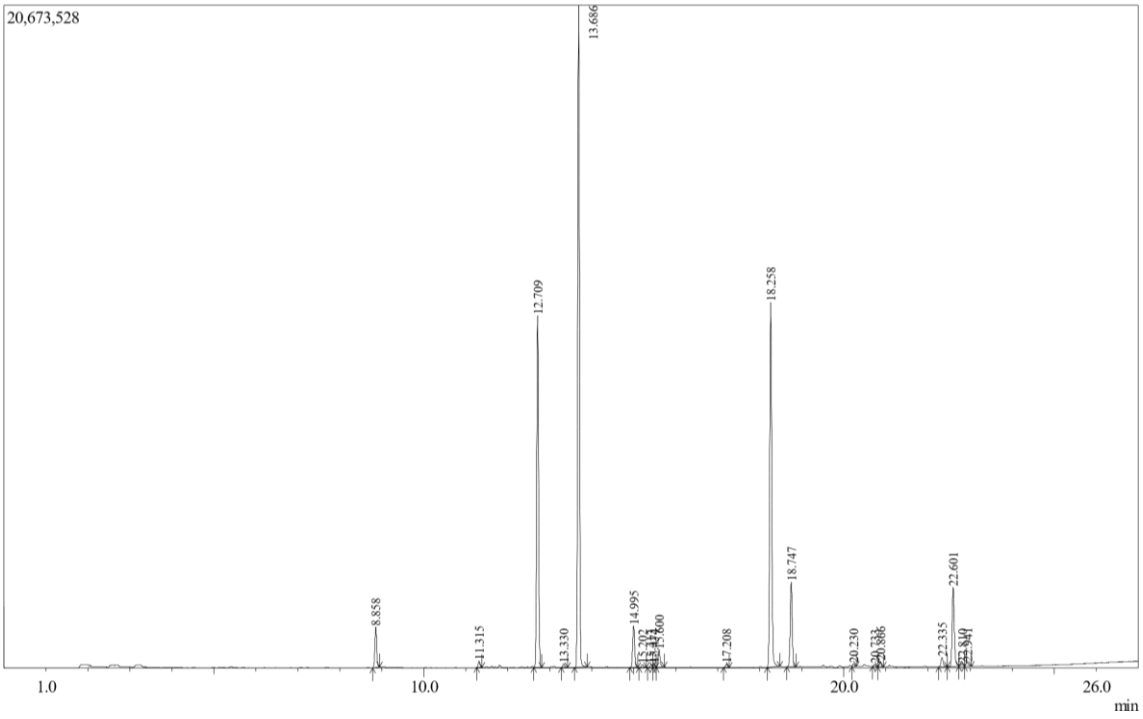
Cellulose Fibre 1 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.855	3640123	1.10	3-Hexen-1-ol, (E)-
2	11.314	512450	0.16	1-Octen-3-ol
3	11.608	634561	0.19	.beta.-Myrcene
4	11.695	630519	0.19	.beta.-Pinene
5	12.579	418836	0.13	D-Limonene
6	12.708	70927122	21.51	Eucalyptol
7	13.329	368348	0.11	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl
8	13.689	136425110	41.36	1,6-Octadien-3-ol, 3,7-dimethyl-
9	14.990	10045699	3.05	(+)-2-Bornanone
10	15.203	974475	0.30	L-.alpha.-Terpineol
11	15.415	1321990	0.40	Terpinen-4-ol
12	15.597	5088111	1.54	.alpha.-Terpineol
13	17.204	379962	0.12	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2
14	18.262	90482673	27.43	Eugenol
15	19.513	357603	0.11	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-me
16	19.700	262683	0.08	.alpha.-Guaiene
17	20.730	563324	0.17	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
18	20.861	822148	0.25	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-met
19	22.336	594611	0.18	cubedol
20	22.603	5366498	1.63	.tau.-Cadinol
		329816846	100.00	

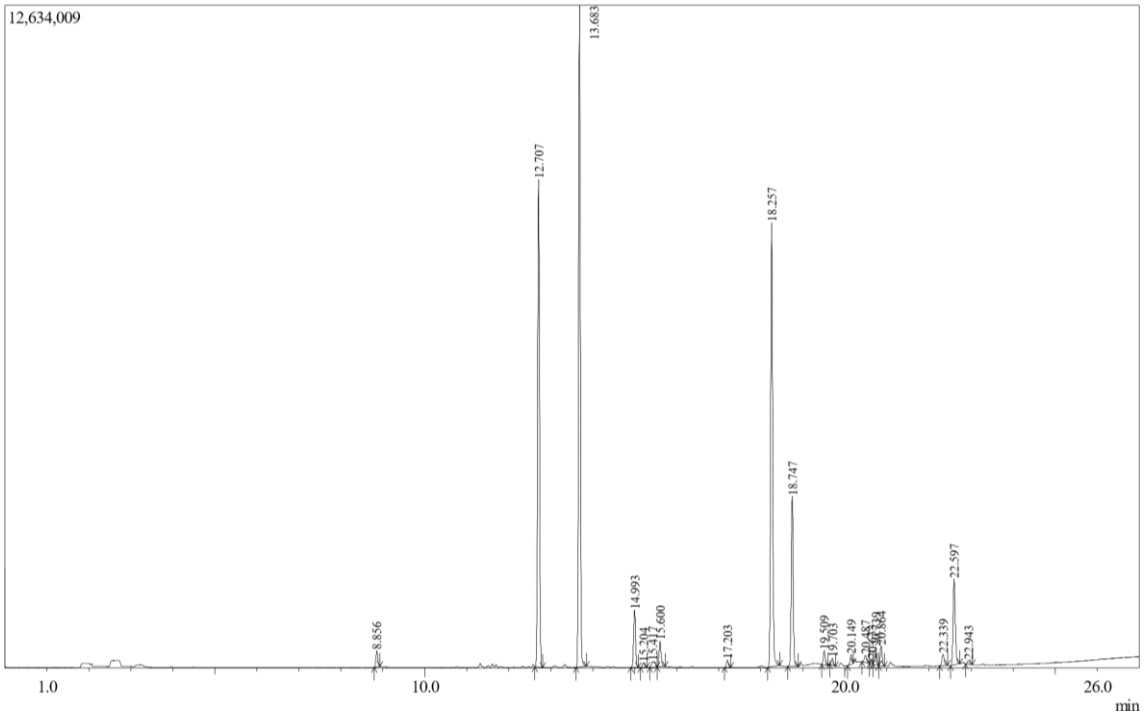
Cellulose Fibre 2 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.858	3780257	2.30	3-Hexen-1-ol, (Z)-
2	11.315	586606	0.36	1-Octen-3-ol
3	12.709	33342740	20.29	Eucalyptol
4	13.330	371830	0.23	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl
5	13.686	60000712	36.50	1,6-Octadien-3-ol, 3,7-dimethyl-
6	14.995	4329848	2.63	Camphor
7	15.202	332105	0.20	.alpha.-Terpineol
8	15.413	320449	0.19	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl
9	15.474	307879	0.19	Acetic acid, octyl ester
10	15.600	1852915	1.13	.alpha.-Terpineol
11	17.208	350576	0.21	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2
12	18.258	37979049	23.11	Eugenol
13	18.747	8773883	5.34	Methyleugenol
14	20.230	245158	0.15	(+)-epi-Bicyclosesquiphellandrene
15	20.733	235406	0.14	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
16	20.866	528454	0.32	.gamma.-Muurolene
17	22.335	1141929	0.69	cubedol
18	22.601	8949117	5.44	.tau.-Cadinol
19	22.810	426605	0.26	.tau.-Cadinol
20	22.941	510852	0.31	Cubenol
		164366370	100.00	

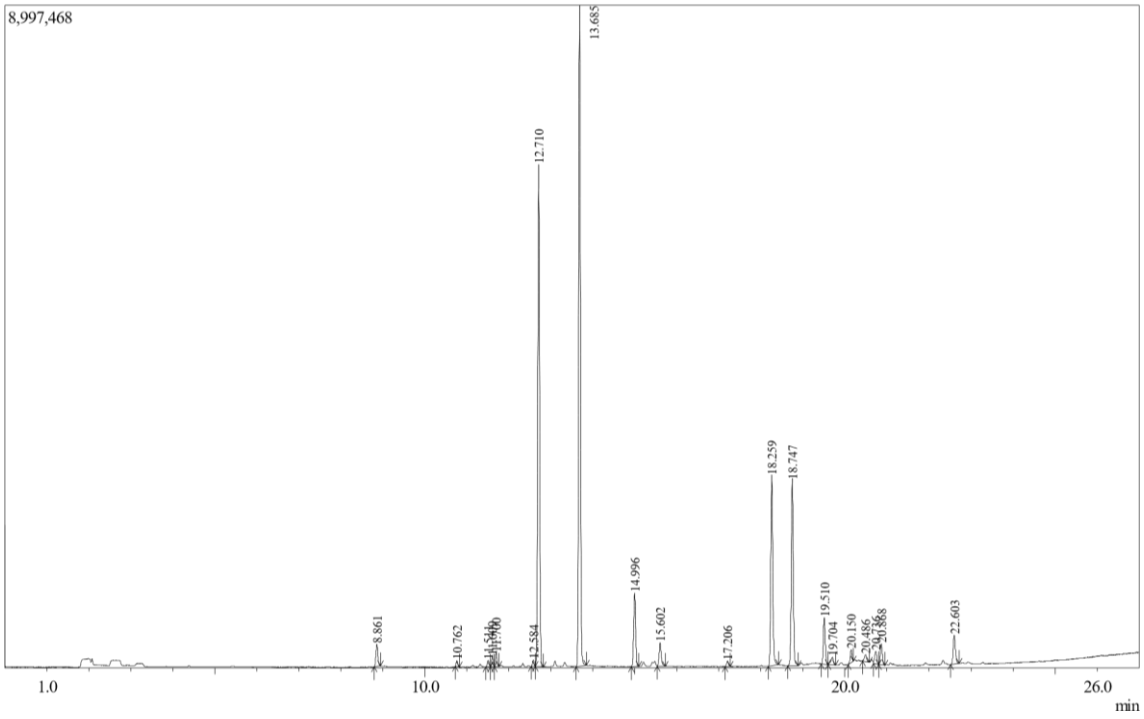
Textile Fibre 1 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.856	951452	0.78	3-Hexen-1-ol, (E)-
2	12.707	28157970	23.08	Eucalyptol
3	13.683	35507472	29.10	1,6-Octadien-3-ol, 3,7-dimethyl-
4	14.993	3561631	2.92	Camphor
5	15.204	289064	0.24	L-.alpha.-Terpineol
6	15.417	621768	0.51	Terpinen-4-ol
7	15.600	1662880	1.36	.alpha.-Terpineol
8	17.203	458851	0.38	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2
9	18.257	27983638	22.93	Eugenol
10	18.747	10626692	8.71	Methyleugenol
11	19.509	861413	0.71	(E)-.beta.-Famesene
12	19.703	374453	0.31	.alpha.-Guaiene
13	20.149	604968	0.50	Humulene
14	20.487	517919	0.42	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1
15	20.633	230024	0.19	Naphthalene, decahydro-4a-methyl-1-methylene
16	20.739	1187165	0.97	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
17	20.864	1256227	1.03	.gamma.-Muurolene
18	22.339	842066	0.69	4-epi-cubedol
19	22.597	6034713	4.95	.tau.-Cadinol
20	22.943	292677	0.24	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.
		122023043	100.00	

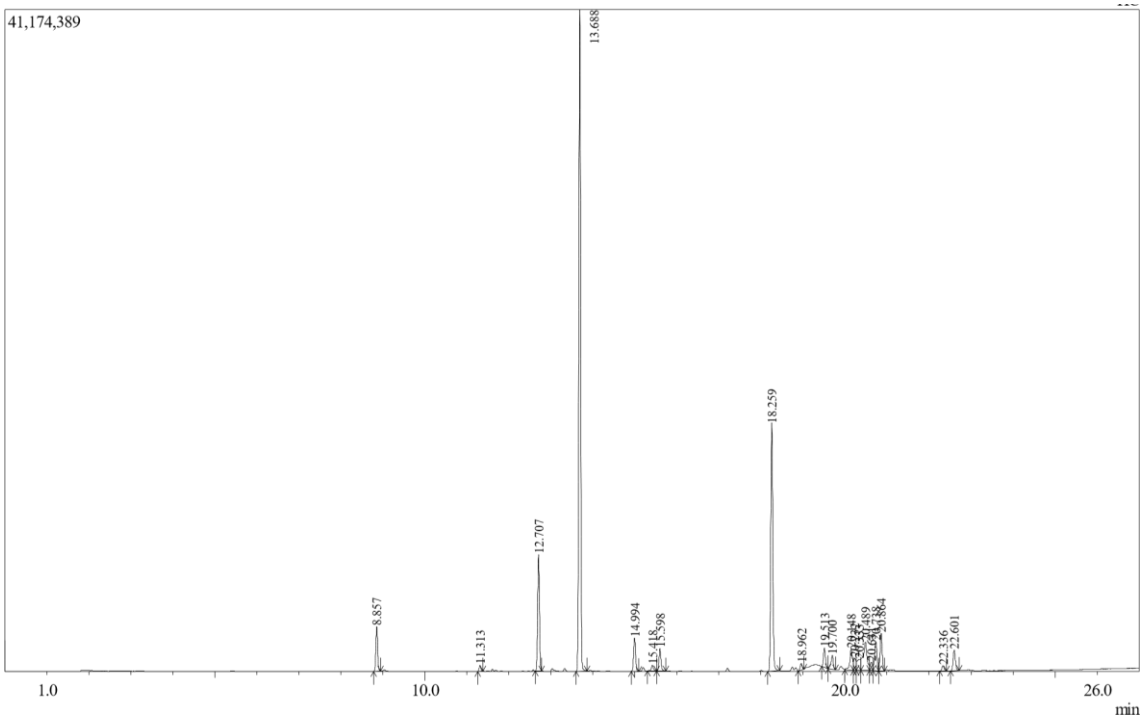
Textile Fibre 2 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.861	964550	1.25	3-Hexen-1-ol, (E)-
2	10.762	218374	0.28	.alpha.-Pinene
3	11.511	255578	0.33	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methyl
4	11.609	361066	0.47	.beta.-Myrcene
5	11.700	533367	0.69	.beta.-Pinene
6	12.584	241523	0.31	D-Limonene
7	12.710	20611642	26.81	Eucalyptol
8	13.685	26077891	33.93	1,6-Octadien-3-ol, 3,7-dimethyl-
9	14.996	3215455	4.18	Camphor
10	15.602	1111617	1.45	.alpha.-Terpineol
11	17.206	229559	0.30	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2
12	18.259	8481719	11.03	Eugenol
13	18.747	8264283	10.75	Methyleugenol
14	19.510	1954544	2.54	cis-.beta.-Farnesene
15	19.704	442058	0.58	.alpha.-Guaiene
16	20.150	525143	0.68	Humulene
17	20.486	331452	0.43	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1
18	20.736	612555	0.80	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
19	20.868	941038	1.22	.gamma.-Muurolene
20	22.603	1493372	1.94	.gamma.-Muurolene
		76866786	100.00	

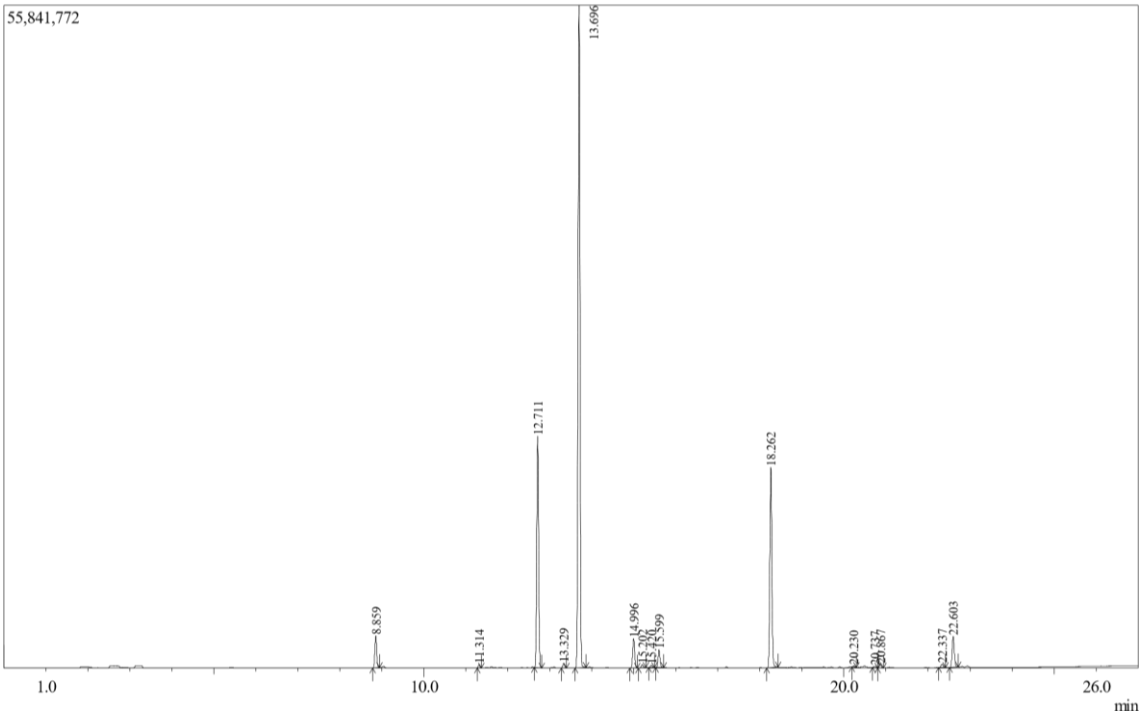
Perlite 1 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.857	8161300	3.02	3-Hexen-1-ol, (Z)-
2	11.313	1015621	0.38	1-Octen-3-ol
3	12.707	21499895	7.95	Eucalyptol
4	13.688	120168109	44.42	1,6-Octadien-3-ol, 3,7-dimethyl-
5	14.994	6717113	2.48	(+)-2-Bornanone
6	15.418	1480887	0.55	Terpinen-4-ol
7	15.598	4592642	1.70	.alpha.-Terpineol
8	18.259	51667981	19.10	Eugenol
9	18.962	1381267	0.51	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-met
10	19.513	3908633	1.44	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-me
11	19.700	3325460	1.23	.alpha.-Guaiene
12	20.148	5725156	2.12	Humulene
13	20.232	2929882	1.08	(+)-epi-Bicyclosesquiphellandrene
14	20.333	3805910	1.41	(E)-.beta.-Famesene
15	20.489	11773229	4.35	.beta.-copaene
16	20.631	2081856	0.77	Naphthalene, decahydro-4a-methyl-1-methylene
17	20.738	6720172	2.48	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
18	20.864	7589967	2.81	.gamma.-Muurolene
19	22.336	1135251	0.42	cubedol
20	22.601	4858299	1.80	.tau.-Cadinol
		270538630	100.00	

Perlite 2 Analysis Report



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.859	8118788	2.43	3-Hexen-1-ol, (Z)-
2	11.314	676760	0.20	1-Octen-3-ol
3	12.711	60617670	18.16	Eucalyptol
4	13.329	1050328	0.31	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl
5	13.696	181003237	54.21	1,6-Octadien-3-ol, 3,7-dimethyl-
6	14.996	8120420	2.43	(+)-2-Bornanone
7	15.202	850756	0.25	L-.alpha.-Terpineol
8	15.420	497958	0.15	Terpinen-4-ol
9	15.599	4818905	1.44	.alpha.-Terpineol
10	18.262	56010459	16.78	Eugenol
11	20.230	295947	0.09	(+)-epi-Bicyclosesquiphellandrene
12	20.737	386910	0.12	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethy
13	20.867	835600	0.25	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-met
14	22.337	1065017	0.32	cubedol
15	22.603	9523107	2.85	.tau.-Cadinol
		333871862	100.00	