The effect of spent mushroom (*Agaricus bisporus*) compost on the indigenous rhizosphere microbiota in strawberry cultivation

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1. Abstract

Spent mushroom compost (SMC) is a by-product of mushroom cultivation with the potential to be used in the cultivation system to suppress plant pathogens, enhance water holding capacity, increase soil water aeration and to improve the soil structure through the input of organic matter and additional nutrients. The electric conductivity (EC) as well as pH recorded high values in the SMC, which is a challenge for its application in food production systems. For the successful use of SMC as a plant growth promoter and diseases suppressive factor, more knowledge about the effect of its amendment to the soil or the growing media is needed. Strawberry cultivation is one of the major production systems within the Swedish Horticultural sector with challenges regarding root pathogens. Suppressive growing media or compost are a strategy of great interest to face this challenge. The current study was carried out to investigate the effect of SMC proportional amendment on the suppressive potential of the growing media and its indigenous rhizosphere microbiota, plant growth, and nutrient content in strawberry cultivation. Five treatments (proportions) with six replicates per treatment were included in the experiment; G1= peat (100%), G2= SMC (100%), G3= SMC (30%): Peat (70%), G4= SMC (50%): Peat (50%) and G5= SMC (70%): Peat (30%). The results give a preliminary understanding of the types of beneficial microbes that occurs in the cultivation system after the amendment of SMC. Utilization of spent mushroom compost enhanced the abundance of nutrient content in the strawberry rhizosphere by increasing the availability of macro and microelements needed for plant growth. It also created a rich microbiota of several microbial groups known for its antagonistic potential such as *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., and *Actinomyces* spp. the highest abundance of microbes was in the G4 treatment except for the *Actinomyces* spp. and *Trichoderma* spp. the G3 treatment was the highest treatment with respect to beneficial effects on plant height and number of leaves. The presence of microorganisms known by their antagonistic properties against plant pathogens and the enzyme activities performed by these microorganisms is an indication of the suppressive effect developed in the growing media after the addition of SMC. The abundance of *Trichoderma* spp. increased by the increase of SMC in the treatments.

Contents

2. Introduction

The increase in the world population accompanied by the decrease of arable lands are challenges facing human food security. Efficient strategies of management to maximize the use of the available resources are thus of great importance. Such a strategy is the implementation and recycling of organic residues in food production. SMC is a by-product of the mushroom cultivation, which could have the potential to enhance plant growth and production by contributing to plant necessary nutrients. It has also a beneficial effect through its abundance of microorganisms that could play an important role in suppressing a wide range of pathogens.

2.1. Spent mushroom compost (SMC)

Mushroom production has increased rapidly in the last few years, and globally the most commonly grown mushrooms are *Agaricus bisporus* "mushroom", *A.subrufrescens* "almond mushroom", *Pleurotus* spp "oyster mushroom", *Lentinula edodes* "shiitake", *Auricularia* spp "wood ear", and *Flammulina* "Enoki" with China as the largest global producer (Royse, 2014). The mushroom (*Agaricus bisporus*) grows on compost, which consists of straw, horse manure, poultry manure, gypsum, and nitrogen-containing compounds such as Urea and water. To produce 1 kg of mushroom 5kg of compost is needed (Paredes et al., 2009).

After the end of harvesting, spent mushroom residues can be reused in organic and conventional farms to enhance the water holding capacity, soil water aeration, and to improve soil structure with organic matter and additional nutrients such as nitrogen, phosphorus, and potassium. Preferably SMC should be aged till 18 months before use due to its high levels of ammonium, water-soluble salt contents, and enable the decomposition of organic matter in the substrate (Ibrahim uzun, 2004).

According to previous studies, the level of nutrients in the dry matter was averaged around 0.8 kg of nitrogen, 3.9 kg phosphorus, and 7.9 kg potassium in each ton of fresh spent mushroom compost. Despite the addition of extra organic nutrient value when mixed with agricultural soil, there is still a challenge of high salinity, high levels of electrical conductivity EC in the growing media, and this fact reported to limit the use of SMC directly as an alternative growing medium or a soil supplement of organic manure. However, the amendment of spent mushroom compost enhances the organic matter and boosts the microbiota biodiversity in the agricultural soils and could probably improve the suppressive characteristics in these soils. (Maher et al, 2000).

2.2. Strawberry cultivation

Swedish strawberry cultivation is a major production for the Swedish market and is considered one of the traditional and cultural fruits in the Swedish society especially in the celebrations of midsummer (Ricard and Ricard, 1997). According to the Swedish Board of Agriculture, the total strawberry cultivation area in Sweden is approximately 2500 hectares with a production of 15400 tons in 2018 (Jordbruksverket 2019). One of the major challenges of strawberry cultivation is the decrease in the quantity and quality of yield due to pathogen attacks. Some examples of pathogens that reduce strawberry yield are:

Phytophthora cactorum causes crown rot disease in strawberry is a challenge in strawberry cultivation and has been known in Europe since 1952, (Deutschmann, V. F. 1954). Several control methods have been applied mitigate the problem, such as the use of chemical fungicides (Aliette 80 WG (fosetyl-Al)), biological control agents and also agricultural practices such as partial resistant cultivars and improved soil drainage (Eikemo et al, 2000, Heil et al, 2000, Parikka, P. 1991, Den Hond, F, 1998, Hammerschmidt, R, 1999)

Phytophthora fragariae causes root rot (red core disease) in strawberry and was discovered by Hickman in 1940(Goode, 1956). *P. fragariae* can survive in the soil for years, even with the absence of the host (Alcock &Howells, 1936).

Early studies mentioned disease suppressive compost as a strategy of disease control due to its content of beneficial microorganisms that suppress various diseases in strawberry cultivation (Bernier-English et al.,2010; Martin- Lapierre et al.,2011). Improving plant's health could happen by suppressive soil which recorded high levels of organic matter, exchangeable Ca, Mg, and N and biological activity (Broadbent and Baker,1974).

2.3. Antagonistic microorganisms

Several microbial groups have been investigated and pointed out for their antagonistic potential against root pathogens. In the current study *Pseudomonas* spp. (Meyer and Abdallah, 1978, Xu et al., 2011), *Bacillus* spp. *(Cloutier et al., 2020)*(Xu et al., 2011), *Actinomyces* spp. (Chen et al., 2008), (Wan et al., 2008) and *Trichoderma* spp. (Hadar and Gorodecki, 1991, Hoitink et al. 1997, Vespermann et al., 2007) are microorganisms in focus.

Pseudomonas spp was widely studied as a bio-fungicide against many pathogens by producing secondary metabolites with antagonistic properties that suppresses different kinds of pathogens like *Phytophthora infestans* and *Phytophthora ramorum (*Meyer and Abdallah, 1978, Xu et al., 2011).

Bacillus subtilis is a bacterium considered one of the biocontrol agents (BCAs) that can suppress different fungal diseases, like *Botrytis cinerea*, *Phytophthora ramorum* (Xu et al., 2011).

Actinomyces is a genus of the *Actinobacteria* class of bacteria, release volatile organic compounds (VOCs) with a high vapor pressure can inhibit pathogens (Vespermann et al., 2007).

Trichoderma harzianum mentioned in many types of researches for its abundance in the mature composts (Hadar and Gorodecki, 1991, Hoitink et al. 1997), *Trichoderma* species were recognized for their potential benefits of suppressing the pathogens as biocontrol agents against plant borne diseases in the 1930s (Weindling, 1932)

Previous studies were done on the mechanisms of the potential suppression of soil-borne diseases included antibiosis, which refers to the release of specific and/or non-toxic specific metabolites or antibiotics by one organism that directly suppresses the activity of pathogens (Cook et al. 1995), microbiostasis, refers to the process of inhibiting the growth, reproduction and multiplication of pathogens but not killing them (Hoitink et al, 1997), competition for nutrients, predation (Curl et al. 1988), parasitism (Harmon 2000), activation of a systemic resistance cascade in host plants (Zhang et al. 1998), and the other abiotic conditions around the root zoon pH, EC, water-holding capacity, micro-nutrients and macro-nutrients (Altomare et al. 1999; Englehard 1989).

The utilization of biocontrol agents (*Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas* spp) is an environmentally friendly strategy to prevent excessive chemical fungicides use against *Phytophthora* spp. and plays a vital role as an alternative or complement to the use of chemical fungicides. However, the biocontrol agents considered one of the best methods in plant disease management programs (Handelsman and Stabb, 1996), and one of the effective biocontrol agents is *Trichoderma harzianum* with a high potential effect on controlling *Phytophthora* spp. (Porras et al., 2007b, Porras et al., 2007a).

Microorganisms breakdown several organic compounds characterized by a complex structure in the organic dry matter. Microorganisms decompose the organic compounds into simple compounds available and absorbable to the plants by different enzymes. Protease catalyzes the proteins into smaller polypeptides or single amino acids, and amylase breaks down amylose and amylopectin into smaller chains of glucose, called dextrins and maltose.

2.4. Microbiota of SMC

The indigenous microbiota of the SMC growing media is relatively unexploited. Potentially antagonistic organisms in the SMC could suppress pathogens in cropping systems and enhance the production by preventing diseases and add some nutrients to the root system of the plants (Akrofi et al., 2017).

Good agricultural and management practices like SMC amendments are needed to initiate and exploit the communities of the resident antagonists in soils. These practices boost the biodiversity which participates in the soil's nutrients richness and save purchase-input of extra charges like chemical fertilizer's needs (Eikemo et al, 2000, Heil et al, 2000, Parikka, P. 1991, Den Hond, F, 1998, Hammerschmidt, R, 1999, Wan et al., 2008).

Earlier studies (Goode, 1956, Meyer and Abdallah, 1978, Xu et al., 2011) referred to some microorganisms in the SMC that could have antagonistic properties against pathogens.

The high contents of salts of the spent mushroom compost could limit its use for the growing salt-sensitive plants like a strawberry; these physical properties could negatively affect the growth of some sensitive plants (Medina et al., 2009).

It could be difficult to determine the SMC mixing ratio with other growing media to enhance the plant's growth media and be economic and sustainable in the strawberry cultivation systems with respect to the high content of microbe's population as well in SMC, despite it was reported that the SMC physical, chemical, and biological characteristics make SMC ideal for blending with other growing media to enhance the growth of plants (Davis et al., 2005).

2.5. Objectives:

This study aims to investigate the effect of the proportional amendments of SMC and peat on the rhizosphere microbiota and plant growth in strawberry cultivation. The following research questions are to be answered:

- What treatments of peat and SMC are to be used to achieve a good effect on both plant growth and microbial parameters in the cultivation system?
- What effect does the addition of SMC have on *Trichoderma* spp. in the system?
- Is there a relationship between the addition of SMC and nutrient content in the growing media?

2.6. Hypotheses:

- The addition of SMC to peat in the treatment of 50:50 is suitable to achieve a good effect on the microbial and growth parameters in the system.
- The growth of microbes known for their antagonistic potential is enhanced by the addition of SMC to peat in 50:50 treatment.
- Growth of *Trichoderma* spp. increases by the increased amount of SMC in the growing media.
- Nutrient content in the growing media increases with an increased amount of SMC.

3. Materials and Methods

3.1. Plant material and substrates

Bare root frigo plants of *Fragaria ×ananassa* cv. Favori were imported from the Netherlands (Flevoplant B.V. Enserweg, 98307, PJ Ens, Holland) and used in the study.'Favori' is an ever-bearing variety, with good shelf life and firm, glossy fruit, good flavor and an early production start. The fruits are conical and elongated in shape; the variety has good resistance against root diseases. 'Favori' was developed and improved from the variety Mara des Bois at Flevo Berry breeding program. The variety can be planted in fields and on raised beds and grow vigorously in growing media in greenhouses as well. The minimum soil temperature suitable for the cultivar is 7-8 ℃, and the outdoor temperature above 10-12 ℃ (Flevoberry 2020).

Peat was bought from a local producing company Hasselfors Garden, Box 1813, 70118 Örebro. *Agaricus bisporus*, SMC was brought from a local mushroom farmer.

3.2. Experimental setup

The study was conducted in the greenhouse facilities at SLU, Alnarp. The plants were cultivated in 1.5-liter pots with one plant in each pot. Five different treatments were used with different treatments of peat (P) and spent mushroom compost SMC (Table 1)); G1= peat (100%), G2= SMC (100%), G3= SMC (30%): Peat (70%), G4= SMC (50%): Peat (50%) and G5= SMC (70%): Peat (30%). Each treatment was tested in six replicates (pots). The pots were placed in a greenhouse chamber for 8 weeks at 20 ºC temperature, 85% humidity, and a photoperiod of 16h light:8h of darkness. The plants were fertilized with 25 ml per pot using SUPERBA™ (8.2 % N + 11,5% P+ 36,1% K+ 2,8% MgO+ TE) fertilizers (NPK, magnesium and micronutrients) twice a week and with 25 ml of CALCINIT (15.5% N + 26.3% CaO), (Calcium nitrate) fertilizer once a weeks.

Table 1*: The treatments used in the experiments: G1= peat (100%), G2= SMC (100%), G3= SMC (30%): Peat (70%), G4= SMC (50%): Peat (50%) and G5= SMC (70%): Peat (30%)*

Treatments	Proportions
G1	Peat (%100)
G2	Spent mushroom compost (%100)
G3	Spent mushroom compost (%30): peat (%70)

G4	Spent mushroom compost (%50): peat (%50)
G5	Spent mushroom compost (%70): peat (%30)

3.3. EC and pH measurements

The EC and pH measurements were conducted on SMC and, peat separately and on the investigated SMC: P treatments as well. The pH and EC measurements were conducted on three different occasions: in the beginning, in the middle, and at the end of the experiment. For measurements of EC and pH, an amount of 200 ml of distilled water was added to 40 g of the investigated treatments in plastic bottles and placed on a rotary shaker for 1 hour. EC and pH were thereafter measured with a Hanna pH / EC / C - mod. Combo (Waterproof). Three replicates were measured from each treatment.

3.4. Plant growth parameters

To determine the effect of the different treatments on the growth and development of the strawberry plants, multiple plant growth parameters were measured. Plant growth parameters were recorded every week during the experiment for the three chosen parameters, plant height, number of leaves per plant, and the length of leaf's petiole.

The height measurements were taken by a ruler and expressed in centimeters from the root to the apex of primary leaves. The number of leaves was counted from each crown of the strawberry plant and leaves marked and expressed as leaf number per plant. The length of a leaf's petiole was recorded for a fixed marked leaf of each strawberry plant and expressed in centimeters.

3.5. Microbial enumeration and analyses

To determine the amount of microbiota in each treatment, samples of a mixture of roots and growing media were analyzed through dilution series and enumeration on selective agar media.

At the end of the experiment, 10 g of roots/growing media were taken from each replicate; 50 ml of detergent was added to each sample. The samples were shaken at 300 rpm for 30 minutes and a serial dilution of sterile 0.85 % NaCl was prepared. Aliquots of 100µl were inoculated on each selective media (Table 2) using a drop plate test. The microbial amounts were estimated by plate count selective media.

***Table 2**: The composition of different types of selective media used in the experiment for microbial enumeration.*

Medium name	Ingredients	Incubation temperature (ºC)	Incubation time/hours
0.1 Tryptic Soya Agar (TSA)	(TSA; DIFCO 0369-17-6) solidified by 1.5% Bacto Agar (DIFCO) and R2A (DIFCO 1826- 17-1), Cycloheximide was added 1 g/100 ml methanol and then 0.1% stock solution was added to the media. For the enumeration of the general bacterial microbiota.	27	48
Malt extract (MA)	Consists of Bacto Malt extract: 10g/l with Bacto Agar: 20g/l (MA, DIFCO 0186-17-7) with Rifampicin 1g/100ml Methanol then 0.1% stock solution was added to the media for the enumeration of the general fungal microbiota.	25	120
Trichoderma-selective medium (TSM)	(K_2HPO_4: 0.9 g/l, $MgSO_4$: 0.2 g/l, KCl: 0.15 g/l, NH_4Cl: 1.05 g/l, Glucose 3 g/l, Rose Bengal: 0.15 g/l, agar 20 g/l, streptomycin: 100 mg/l, tetracycline: 50 mg/l), for the enumeration of *Trichoderma* spp.	25	120
Nutrient Agar (N.A)	(Peptic digest of animal tissue: 5 g/l, NaCl:5 g/l, beef extract: 1.5 g/l, yeast extract: 1.5 g/l, agar: 20g/l) for the enumeration of *Bacillus* spp.	27	24
King's B agar (KB)	(Peptone: 20g/l, K_2HPO_4: 1.5 g/l, MgSO4: 1.5 g/l, glycerol: 10 ml/l, agar: 20g/l) 28. For the enumeration of *Pseudomonas* spp.	27	48
Modified Bennett's medium (MB)	17117 *Actinomycete* Isolation Agar was used for the enumeration of *Actinomyces* spp.	27	48

3.6. Enzyme activity

The minimal media (M9) contains the minimum nutrients possible for colony growth without the presence of amino acids (Appendix 1) was used for the enzyme activity assessment.

Pre-setup for enzyme studies, one distinctive colony from each agar medium was picked with a disposable inoculation loop and spread onto either full strength TSA for general bacterial flora, King agar media (KB) for *Pseudomonas* spp or Potato Dextrose Agar (PDA) for general fungal flora using the quadrant streak method. The plates were incubated at 25 ºC for 24 hours. After the pure culture procedure, one colony from each media was thereafter inoculated in 5 ml of tryptic soy broth (TSB) for the general bacterial flora, KB broth (KBB) for *Pseudomonas* spp or Potato Dextrose broth (PDB) for general fungal flora and incubated at 25 ºC for 24 hours. The cultures were then spread on different special enzymatic media as described below two replicates for each growing media. In total 144 isolates were investigated (appendix 2).

3.6.1. Protease activities

Bacterial isolates from pure culture were used to identify bacterial protease activities. M9 minimal media agar amended with skimmed milk (20 ml/l). The spot technique inoculated the plates with the bacterial isolate and incubated at 20 ºC for 72 hours. A formation of a clear halo around the bacterial colonies is considered a positive result. (Choudhary et al, 2009)

3.6.2. Amylase activities

The bacterial isolates from the pure culture inoculated on prepared plates of M9 minimal media agar amended with Trichoderma xylanase (1% w/v) media by spot inoculate technique, the plates were incubated at 20ºC for 72 hours and thereafter flooded with Congo red solution (0, 2% w/v) for 30 min. Excess reagent was discarded after detaining with 1M NaCl for 30 min. Recognizing a zone of clearance around the bacterial colonies was considered a positive result of the amylase enzyme activity (Choudhary, Agarwal, and Johri, 2009).

3.6.3. Organic Phosphorous solubilization

Spot inoculating the isolates from a bacterial pure culture on prepared Tryptose agar plates supplemented with methyl green (0.05 mg ml^{-1}) as indicator dye was performed. The plates were then incubated in an incubator at 20 ºC for 3-5 days. The development of green color by the bacterial colonies was considered a positive result (Choudhary, Agarwal, and Johri, 2009).

3.7. Nutrient analyses

Nutrient analyses were performed on both pure SMC as well as the treatments. An amount of 10g of pure SMC was sent at the beginning of the experiment for the nutrient analyses to the local laboratory LMI AB, Sweden (https://www.lmiab.com)

A sample of 10g from each replicate of the different treatments was also sent to LMI AB at the harvest time.

3.8. Biomass analyses

To estimate the plant biomass with respect to the treatments, a sample of roots and leaves were taken from all the treatment replicates, the fresh weight of roots and leaves were recorded, then dried in drying cabinet 90 ºC for 3-5 days, finally, the total weight was recorded, and the dry net weight was calculated for every replicate in the experiment.

3.9. Statistics

Statistics were subjected to the method analyses of variance (ANOVA) using the statistical software package, Minitab®19 from Minitab, Ltd. Significance ($P \leq 0.05$), treatment means were separated using Tukey method significant difference test (α level = 0.05).

IBM SPSS Statistics software from International Business Machines Corporation (IBM) was used to run the correlations among variables, significance ($P \leq 0.01$), and ($P \leq 0.05$).

4. Results

Strawberry plant growth was severely limited in the G5 treatment, and partly in G4. Hence the following results present the treatments of G1, G2, and G3 and three replicates out of originally six replicates in G4 treatment.

4.1. EC and pH measurements

The pH value in G1 treatment was suitable and similar the pH value required for strawberry growth pH requirements when measured at the beginning of the experiment and recorded 6.53, in the middle recorded 6.53, and at the end of the experiment recorded 6.43. The pH of G2 and G4 treatments showed lower pH values at the end of the experiment and recorded 6.87, 7.79 respectively compared to pH values at the beginning 8.8, 8.2, respectively. The G5 treatment recorded pH values of 7.1 at the middle of the experiment while pH values recorded 8.4 at the beginning of the experiment. The G3 treatment was the only treatment that showed a slight increase in pH between the beginning, 6.43, and the end, 7.1 (Table 3).

The use of a stock solution of phosphoric acid reduced the pH to suitable values in all treatments of SMC: Peat at the beginning of the experiment (Figure 1).

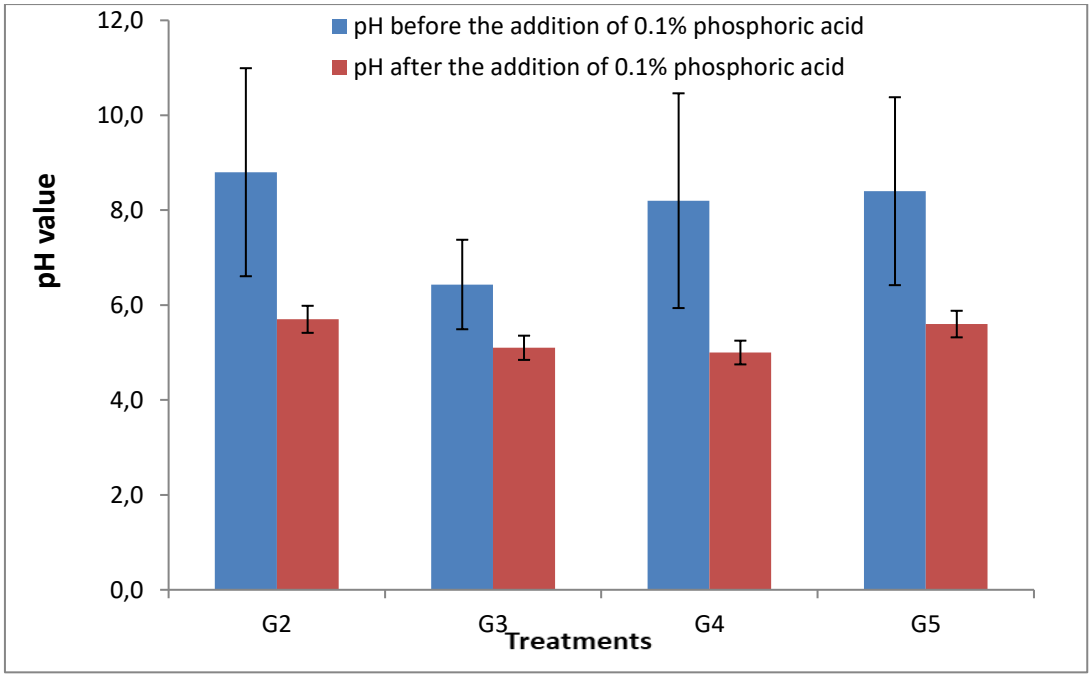

Figure 1: The pH readings in the treatments, G1 = peat (100%), G2 = SMC (100%), G3= SMC (70%): Peat (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) at the beginning of the experiment with and without the addition of 0.1% phosphoric acid.

***Table 3**: The pH values as effected by the treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), and G5 = SMC (30%): Peat (70%) measured at the beginning, in the middle and at the end of the experiment. Means that do not share the same letter are significantly different based on Tukey's test at $P \leq 0.05$* within column.

Treatments	Beginning of the experiment	Middle of the experiment	End of the experiment
G1	6,53 ^{c}	6,53 ^{c}	6,433 ^{c}
G2	8,8 ^{a}	7,46 ^{a}	6,87^{bc}
G3	6,43 ^{c}	7,06 ^{b}	7,1 ^{b}
G4	8,2 ^{b}	7,2 ^{b}	7,79 ^{a}
G5	8,4 ^{b}	7,133 ^{b}	

The EC recorded very high values in G2, G3, and G4 at the beginning of the experiment and increased later to settle down on fairly strawberry's growth values. The EC values showed high variance both between time points and between growing media, as the lowest measured value only showed 30µS/cm in G1 and the highest measured value showed 3366.66 µS/cm in G2 in the middle of the experiment. The highest EC was indicated in all the treatments during the middle time of the experiment, and the lowest EC was indicated in all the treatments during the end time, with G1 being the exception. The treatment G1 showed the highest measured value at the last time point but also showed significantly lower overall EC values compared with the other treatments. The treatments G2-G5 showed EC values between 1000-3000 µS/cm for most time points measured, while the treatment G1 showed an EC value of only 106.66 µS/cm at the highest time point measured (Beginning, middle and the end of the experiment). The treatment G1 showed the lowest overall EC value and G2 treatment showed the overall highest EC value (Table 4).

***Table 4**: The EC µS/cm as effected by treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) measured at the beginning, in the middle and at the end of the experiment. Means that do not share the same letter are significantly different based on Tukey's test at $P \leq 0.05$ within column.*

Treatments	Beginning of the experiment	Middle of the experiment	End of the experiment
G1	50 ^{d}	30 ^{d}	106,66 ^{c}
G2	2766,66 ^{a}	3366,66 ^{a}	2300 ^{a}
G3	1120 ^{c}	1680 ^{c}	593,33 ^{b}
G4	1693,33 ^{b}	1903,33 ^{c}	890 ^{b}
G5	1600 ^{b}	2733,33 ^{b}	

4.2. Nutrients content in pure SMC

The pure SMC had a C:N ration of 21.7 mg/kg. The nutrient analyses indicated also high content of the macro elements Ca, Mg, Na, S, and K (Table 5).

Table 5*: The SMC nutrient content analyses at the beginning of the experiment by LMI AB, Sweden.*

Subject	Result (mg/kg)
C/N	21,7
Total-C	83400
Total-N	3840
Al	13
B	1,7
Ca	10500
Cd	< 0,08
Cu	0,56
Fe	20
K	4700
Mg	961
Mn	43
Mo	< 0,12
Na	1200
Ni	< 0,30
P	853
S	3100
Si	78
Zn	21

4.3. Plant growth parameters

The influence of SMC with respect to the strawberry's plant height showed that G1treatment was statistically (P-value = 0.000) the highest plant height at the end of the experiment followed by G3, and G2. The lowest plant height (P-value = 0.000) was indicated in G4 (Figure 2) compared with the other treatments.

The G3 treatment recorded at end of the experiment the significantly (P-value = 0.015) highest number of leaves compared to the other treatments followed by G2. However, no significant differences with respect to the number of leaves were indicated in the other treatments (Figure 3).

At the end of the experiment, the G1 treatment indicated the highest plant length (P-value = 0.007) with respect to leaf's petiole followed by G2 and G4 while G3 was not significant with respect to leaf's petiole (Figure 4).

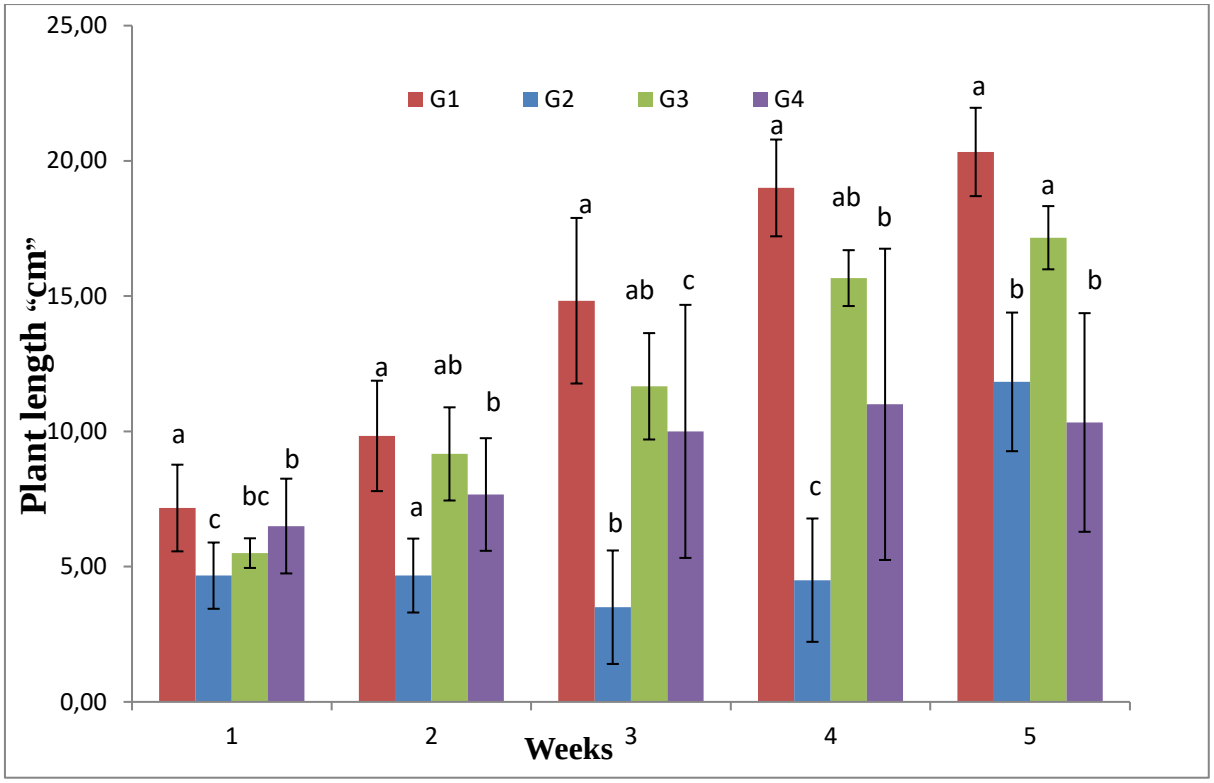

Figure 2: The effects of the G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) on the height of five-weeks old strawberry plants. Vertical error bars denote standard deviation. Means that do not share the same letter are significantly different based on Tukey's test at $P \leq 0.05$.

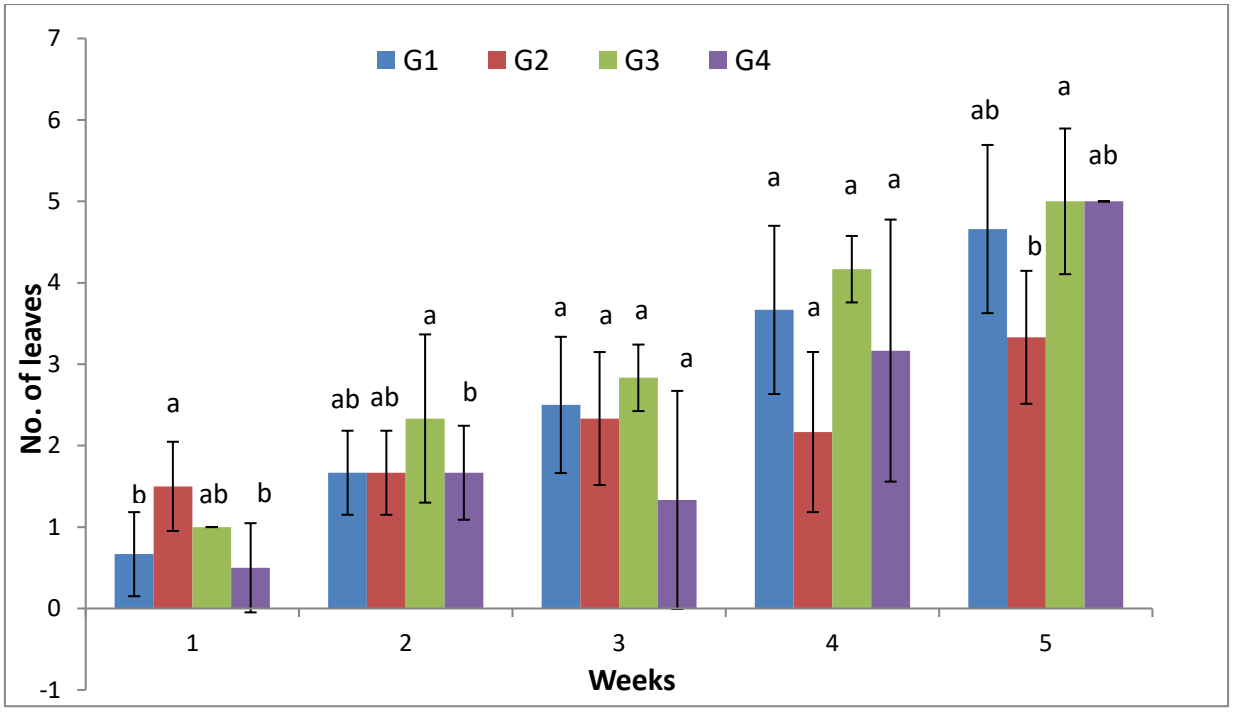

Figure 3: -The effects of SMC: Peat treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) on the number of leaves of five-weeks old strawberry plants. Vertical error bars denote standard deviation. Means that do not share the same letter are significantly different based on Tukey's test at $P \leq 0.05$.

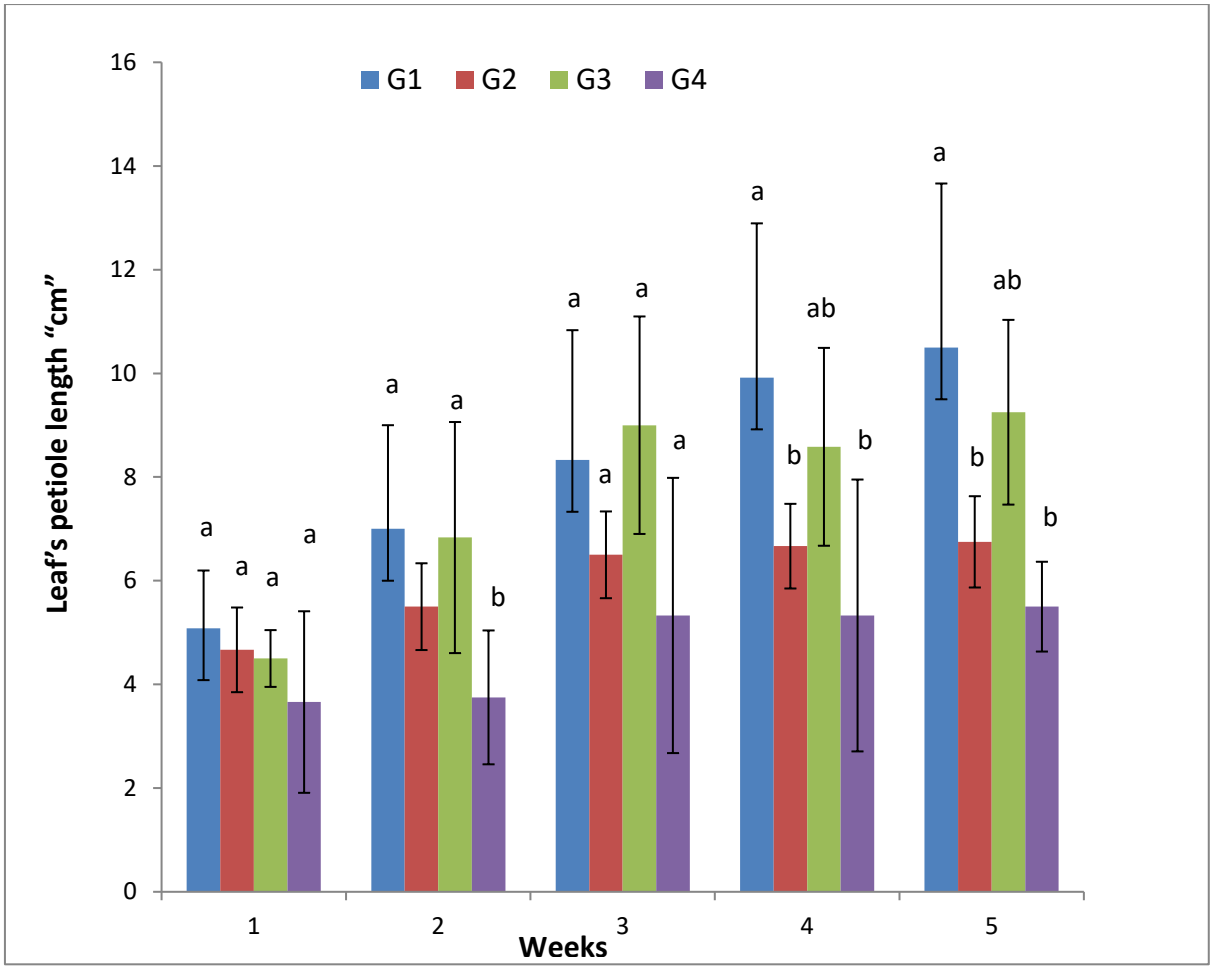

Figure 4: The effects of the treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) on the leaf's petiole of five-weeks old strawberry plants. Vertical error bars denote standard deviation. Means that do not share the same letter are significantly different based on Tukey's test at $P \leq 0.05$.

4.4. The microbial enumeration and in the microbiota of pure SMC (100%) at the start and the end of the experiment

With respect to pure SMC and it´s microbial content, it was found that there was an increase in the abundance of *Pseudomonas* spp., general fungi, and *Trichoderma* spp. at the end of the experiment compared with their abundance at the beginning. Both the general bacterial biota and *Bacillus* spp. recorded a decrease in their abundance at the end of the experiment compared with the abundance at the beginning of the experiment (Figure 5).

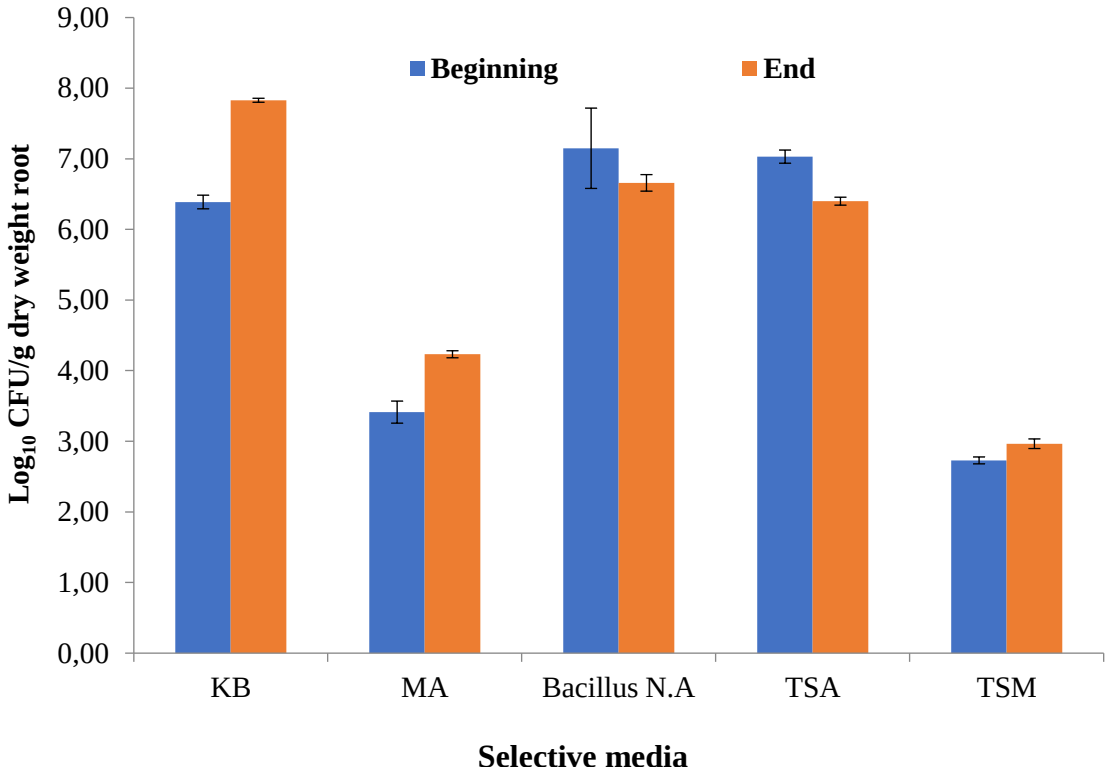

Figure 5:-The amount of microorganisms in Log_{10} CFU/g in SMC (100%) inoculated and enumerated on selective media: King B agar (KB) for enumeration of *Pseudomonas* spp., malt extract agar (MA) for enumeration of general fungi, Nutrient agar (N.A) for enumeration of *Bacillus* spp., tryptic Soy agar (TSA) for enumeration of the general bacteria, Trichoderma selective media (TSM) for enumeration of *Trichoderma* spp.) at the beginning and the end of the experiment. Vertical error bars denote standard deviation.

4.5. The microbial enumeration in the growing media and roots in the treatments based on the used SMC: peat proportions.

The highest amount of *Trichoderma* spp. was found in G2 while the lowest in G1 and the difference was statistically significant in the TSM selective media (P-value = 0,012) (Figure 6).

The highest amount of *Actinomyces* spp. was found in G3 treatment and the less in G1 treatment while the lowest in G2 treatment and the difference was statistically significant (P-value = 0,000) in the MB selective media (Figure 6).

The highest amount of *Pseudomonas* spp. was found in G2, G3 and G4 treatments with no significant differences between them and the less in G1 treatment and the differences were statistically significant (P-value = 0,000) in the KB selective media (Figure 6).

The highest amount of general fungi Was found in both G1and G4 treatments with no significant differences between them and the less in G3 treatment and the least in G2 treatment and the difference was statistically significant (P-value = 0,000) in the MA selective media (Figure 6).

The highest amount of general bacteria Was found in G1 treatment, and the less in G4 treatment and the least in G2 treatment and the difference was statistically significant while G3 treatment was not statistically different compared to the other three growing media (P-value = 0,000) in the TSA selective media (Figure 6).

Both G1 and G4 treatments seemed to contain a high amount of microorganisms compared to the other growing media as they contained high levels of general fungi, general bacteria, and *Bacillus* spp.

G2 seemed to contain lower amounts of microorganisms compared to the other growing media as it contained the least amount of general fungi, general bacteria, and *Actinomyces* spp., and the second to least amount of *Bacillus* spp. G3 seemed to contain an average amount of the microorganisms compared to the other three growing media.

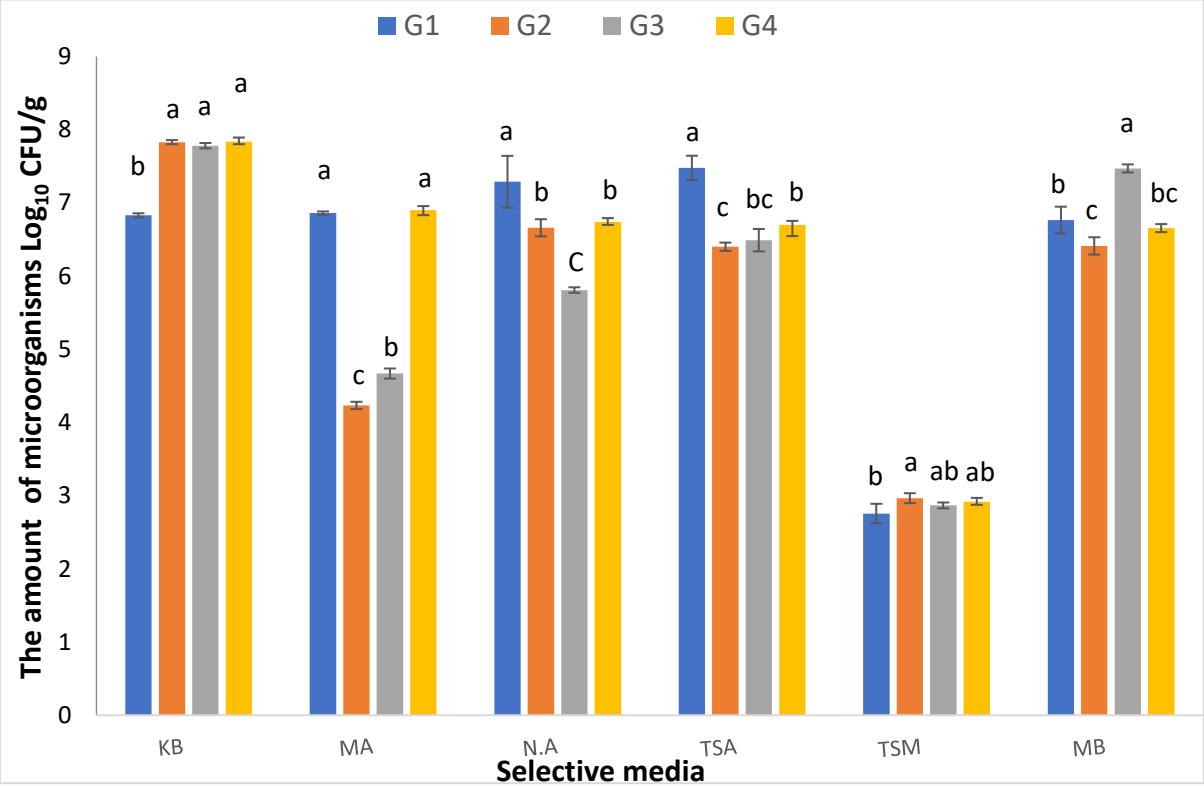

Figure 6: The effects of the treatments on the amount of the microbiota in Log_{10} CFU/g in the experiment using the SMC: peat proportions G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) inoculated and enumerated on selective media: King B agar (KB) for enumeration of *Pseudomonas* spp., Malt extract agar (MA) for enumeration of the general fungal, Nutrient agar (N.A) for enumeration of *Bacillus* spp., Tryptic Soy agar (TSA) for enumeration of the general

bacterial flora, Trichoderma selective media (TSM) for enumeration of *Trichoderma* spp.), and Modified Bennett's medium (MB) for the enumeration of *Actinomyces* spp. at the end of the experiment. Vertical error bars denote standard deviation. Means that do not share the same letter are significantly different based on Tukey's test at $P \leq 0.05$.

4.6. Enzyme assessment

The 144 isolates tested and expressed positive for protease, amylase, and phosphorous solubilization (appendix 2, 3, 4, 5).

4.7. Nutrient analyses at the end of the experiment

4.7.1. Macro-elements content

G2 and G4 contained statistically the highest content of nitrogen compared to G1 which contained the least while G3 had less than G2 and G4.

With respect to phosphorus content G2 contained the highest value while G3 has less and the G1 recorded the least, G4 was not statistically different compared to the other three growing media.

Most of the other macro elements recorded the highest content in G2 followed by G3 and G4 while the G1 recorded the least content of K, S, Ca, while Mg was the same levels in the treatments (Table 7).

Table 7*: The content of macro-elements by mg/l in the treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) at harvest time. Means that do not share a letter are significantly different based on Tukey's test at P ≤ 0.05.*

Treatments	N	P	K	Mg	S	Ca
G1	1,71 ^{c}	40,16 ^{c}	48,3 ^{c}	160 ^{a}	15,3 ^{b}	998,3 ^{c}
G2	225 ^{a}	538,3 ^{a}	2050 ^{a}	476,6 ^{a}	1533,3 ^{a}	3350 ^{a}
G3	102 ^{b}	345 ^{b}	808,3 ^{b}	400 ^{a}	148,3 ^{b}	1516,6 ^{b}
G4	173,3 ^{a}	390,0^{ab}	1266,6 ^{b}	266,6 ^{a}	190 ^{b}	1766,6 ^{b}

4.7.2. The content of micro-elements content

Na and Cl were statistically (P-value = 0,000) significantly higher in G2 followed by G3 and G4 while G1 recorded the least in contents.

Cu was the highest in G2 statistically different and followed by G4 and G1 while G3 was non-significant the least content of Cu (Table 8).

Table 8*: The micro-elements in mg/l in the treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%) at harvest time. Means that do not share a letter are significantly different based on Tukey's test at P ≤ 0.05.*

Treatments	Mn	B	Cu	Fe	Zn	Mo	Na	Cl	Al
G1	0,7 [b]	0,1[d]	0,8 [c]	0,7[ab]	3,5 [c]	0,3 [a]	55 [c]	41,3 [c]	0,6 [a]
G2	1,4 [a]	1,4 [a]	3,1[a]	0,6 [b]	44,8 [a]	0,2[ab]	261, [a]	395 [a]	0,2 [b]
G3	1,1[ab]	0,6 [c]	1,5[bc]	0,8 [a]	16,6 [b]	0,2[ab]	136,6 [b]	151,6 [b]	0,4 [a]
G4	1,3[ab]	0,9 [b]	2 [b]	0,6[ab]	22,3 [b]	0,1 [b]	176,6 [b]	213,3 [b]	0,4 [a]

4.7.3. C:N ratio

G1 contained statistically (P-value = 0,000) the highest ratio of C:N. G3 and G4 contained less, and G2 contained the lowest ratio of C:N (Table 9).

Table 9*: C:N, total N and total C values mg kg^{-1} at harvest time for the treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%). Means that do not share the same letter are significantly different based on Tukey's test at P ≤ 0.05.*

Treatments	C:N	Total N mg per kg	Total C mg per kg
G1	37,2 [a]	12400 [c]	461666,6 a
G2	12,5 [d]	18933,3 [a]	238500 [c]
G3	21,7 [b]	16466,6 [b]	358833,3 [b]
G4	16,8 [c]	16566,6 [b]	245705,5 [c]

4.8. The biomass analyses results

The highest dry matter was observed statistically significant in G1 followed by G2, G4 respectively and G3 was not statistically significant compared to the other growing media (Table 10).

Table 10*: Measurements of the dry matter of strawberry plants for the treatments G1 = peat (100%), G2 = SMC (100%), G3 = Peat (70%): SMC (30%), G4 = SMC (50%): Peat (50%), G5 = SMC (30%): Peat (70%).Means that do not share a letter are significantly different based on Tukey's test at P ≤ 0.05.*

Treatments	Fresh weight leaves (g)	Fresh weight Roots (g)	Dry weight leaves (g)	Dry weight Roots (g)	Total fresh weight (g)	Total dry weight (g)
G1	11,6 [a]	12,2 [a]	3,1 [a]	5,9 [a]	23,8 [a]	8,9 [a]
G2	6,8 [b]	5,36 [b]	1,9 [a]	2,2 [b]	12,1 [b]	4,2 [b]
G3	13,1 [a]	9,5[ab]	2,8 [a]	3,5[ab]	22,6 [a]	6,3[ab]
G4	7,7[ab]	6,7[ab]	1,8 [a]	1,9 [b]	14,5[ab]	3,7 [b]

4.9. Relationship between nutrient content, abundance of the microorganisms and growth parameters.

Different correlations were recorded in our study between the nutrients and abundance of the microorganisms in the root area of strawberry.

A strong significant positive relationship between the concentration of macroelements N, P, and K recorded r= 0.723, r= 0.603, r= 0.742 respectively appeared clearly with the abundance of *Trichoderma* spp. at significance $P \leq 0.01$ (Table 11).

***Table 11**: The relationship between the N, P, and K nutrients, and the amount of Trichoderma* spp. *in the microbiota of strawberry at the end of the experiment.*

		N mg/l	P mg/l	K mg/l
Amount of *Trichoderma* spp. $\log_{10}$ CFU/g	Pearson Correlation	.723**	.603**	.742**
	Sig. (2-tailed)	.000	.004	.000
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				

A strong significant positive relationship between the concentration of macroelements N, P, and K recorded r= 0.819, r= 0.863, r= 0.796 respectively appeared clearly with the abundance of *Pseudomonas* spp at significance $P \leq 0.01$ (Table 12).

***Table12**: The relationship between the N, P, and K nutrients, and the amount of Pseudomonas* spp *in the microbiota of strawberry at the end of the experiment.*

		N mg/l	P mg/l	K mg/l
Amount of *Pseudomonas spp* $\log_{10}$ CFU/g	Pearson Correlation	.819**	.863**	.796**
	Sig. (2-tailed)	.000	.000	.000
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				

A strong significant negative relationship between the concentration of macroelements N, P, and K recorded r= -0.528, r= -0.625, r=-0.508 respectively appeared clearly with the abundance of *Bacillus* spp. at significance $P \leq 0.05$ and $P \leq 0.01$ (Table 13).

***Table 13**: The relationship between the N, P, and K nutrients, and the amount of Bacillus* spp. *in the microbiota of strawberry.*

		N mg/l	P mg/l	K mg/l
Amount of *Bacillus* spp. $\log_{10}$ CFU/g	Pearson Correlation	-.528*	-.625**	-.508*
	Sig. (2-tailed)	.014	.002	.019
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

A strong significant negative relationship between the concentration of macroelements N, P, and K recorded r= -0.722 r= -0.801, r=-0.712 respectively appeared clearly with the abundance of general bacteria on the TSA medium at significance $P \leq 0.01$ (Table 14).

***Table 14**: The relationship between the N, P, and K nutrients, and the amount of general bacteria in the microbiota of strawberry at the end of the experiment.*

		N mg/l	P mg/l	K mg/l
Amount of general bacteria log_{10} CFU/g	Pearson Correlation	-.722**	-.801**	-.712**
	Sig. (2-tailed)	.000	.000	.000
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				

A strong significant negative relationship between the concentration of macroelements N, P, and K recorded r= -0.509, r= -0.621, r=-0.551 respectively appeared clearly with the abundance of general fungi on the MA medium at significance $P \leq 0.05$ and $P \leq 0.01$ (Table 15).

***Table 15**: The relationship between the N, P, and K nutrients, and the amount of general fungi in the microbiota of strawberry at the end of the experiment.*

		N mg/l	P mg/l	K mg/l
Amount of general fungi log_{10} CFU/g	Pearson Correlation	-.509*	-.621**	-.551**
	Sig. (2-tailed)	.019	.003	.010
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

A weak non-significant negative relationship between the concentration of macroelements N, P, and K recorded r= -0.194, r= -0.016, r=-0.260 respectively appeared clearly with the abundance of *Actinomyces* spp. (Table 16).

Table 16: The relationship between the N, P, and K nutrients, and the amount of *Actinomyces* spp. in the microbiota of strawberry at the end of the experiment.

		N mg/l	P mg/l	K mg/l
Amount of *Actinomyces* spp. log_{10} CFU/g	Pearson Correlation	-.194	-.016	-.260
	Sig. (2-tailed)	.400	.945	.254
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				

A strong significant negative relationship between the concentration of macroelements N, P, and K recorded r= -0.783, r= -0.747, r=-0.768 respectively appeared clearly with the plant height at significance $P \leq 0.01$ (Table 17).

***Table 17**: The relationship between the N, P, and K nutrients, and the plant height of strawberry at the end of the experiment.*

		N mg/l	P mg/l	K mg/l
Plant height "cm"	Pearson Correlation	-.783**	-.747**	-.768**
	Sig. (2-tailed)	.000	.000	.000
	N	21	21	21
**. Correlation is significant at the 0.01 level (2-tailed).				

A strong significant positive relationship between the concentration of Cu and the amount of *Trichoderma* spp. and *Pseudomonas* spp recorded r= 0.491, r= 0.658, while other microorganisms relationship with Cu nutrient recorded non-significant negative relationship at *Bacillus* spp. and *Actinomyces* spp. r= -0,431, r= -0,334 respectively and a strong significant negative relationship at general fungi and general bacteria r= -0,516, r= -0,583 respectively (Table 18).

***Table 18**: The relationship between the Cu, and the amount of Trichoderma* spp.*, Pseudomonas* spp.*, Bacillus* spp.*, Actinomyces* spp.*, general fungi, and general bacteria in the microbiota of strawberry at the end of the experiment.*

		Cu mg/l
Amount of *Pseudomonas* spp. log_{10} CFU/g	Pearson Correlation	0.658**
	Sig. (2-tailed)	0.001173261
	N	21
Amount of general fungi log_{10} CFU/g	Pearson Correlation	-0.516*
	Sig. (2-tailed)	0.016687135
	N	21
Bacillus/N.A Log10 CFU/g	Pearson Correlation	-0.431655003
	Sig. (2-tailed)	0.050711512
	N	21
Amount of general bacteria log_{10} CFU/g	Pearson Correlation	-0.583**
	Sig. (2-tailed)	0,005524996
	N	21
Amount of *Trichoderma* spp. log_{10} CFU/g	Pearson Correlation	0.491*
	Sig. (2-tailed)	0.0237392

	N	21
Amount of *Actinomyces* spp. $\log_{10}$ CFU/g	Pearson Correlation	-0.3347162
	Sig. (2-tailed)	0.138045478
	N	21
**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).		

Data of other correlations between microelement concentration and growth parameters can be found in (Appendix 6).

5. Discussion

In this study, spent mushroom compost (SMC) was tested alone or mixed with peat in different treatments to investigate its effect on the indigenous rhizosphere microbiota and growth of cultivated strawberry grown in greenhouses.

The results indicate that SMC could be used in a certain proportion with peat as an organic amendment that enhances nutrients availability and plant growth. Moreover, the richness of microorganisms in these proportions which can play an important role in biodegradation and releasing nutrients, some promoting and antagonistic materials which could suppress some kind of diseases and promote the growth of strawberry plants.

The pH recorded alkaline values (pH= 8.8) and high in the treatment with SMC 100% (G2), or SMC mixed with peat in the proportions of 50%:50% (G4) (pH= 8.2), and (G5) 30%:70% (pH= 8.4) at the beginning of the experiment. The high levels of pH in SMC were reported by Wang et al. (1984). The pH parameter was a challenge for the growth of the strawberry plants, adjusting pH in G2, G3, and G4 treatments by the phosphoric acid at the beginning of the experiment calibrated the pH for the plant's growth by considering the optimal pH levels 5.5-6.5 for nutrient availability in general. The decrease of pH could be happened by the result of adding the phosphoric acid at the beginning of the experiment or the activities of the microorganisms at the end of the experiment. Low pH increases the solubility of some minerals Al, Mn, and Fe to reach high levels of availability and could be toxic to the plants, and high pH makes difficulties to the mineral solubility, and plant absorption which negatively affects the growth of plants (Wortman, 2015).

The result indicates that G1 treatment has a stable chemical composition that does not promote the release of nutrients, as a stable pH indicates that no nutrients have been taken up by the strawberry plants. This could be compared to the G2, G4, and G5 treatments, which all showed declining pH values, indicating that the strawberry plants in these media have higher accessibility to nutrients. This also indicates that reducing the amount of SMC in a growing medium improves nutrient uptake. G3 treatment recorded a significant lowest value of pH compared with G2 and G4 treatments at the end of the experiment, which makes it the proper growing medium with respect to the pH.

The EC was high around 2700 µS/cm in G2 and probably affected plant growth and increased by the time caused to the high contents of Ca, K, Na, S, Mg, and P in the crude SMC and had high values in the SMC mixtures, these high values reported in Wang et al (1984). The high value of EC could have negative effects on the strawberry which considered a salt-sensitive plant and its optimal EC level is 1000–2000 µS/cm for strawberry plants (IPM for Strawberries, 2008). G3 recorded a significant lowest value of EC compared with G2 and

G4 at the end of the experiment, which makes it the proper growing medium with respect to EC.

According to our findings in the experiment, G3 treatment could be optimal in growth readings since it recorded significant high values with respect to height and number of leaves parameters comparing with G2 and G4 treatments at the end of the experiment. Besides G3 treatment recorded the lowest pH and EC values, high content of the dry matter, and contained an average amount of microorganisms compared to G2 and G4 treatments. So G3 treatment is the treatment to be used to achieve a good effect on both plant growth, and microbial parameters in the cultivation system, which covers our first research question.

The G4 treatment recorded significantly high content of microorganisms comparing with G2 and G3 treatments for all tested microbes except *Actinomyces* spp and *Trichoderma* spp. at the end of the experiment, and could be considered as the best-suggested treatment of spent mushroom compost with peat for the microbial activities in the strawberry cultivation and support our hypothesis.

Trichoderma spp. populations were the highest in the G2 treatment in the strawberry cultivation system mentioned in previous researches (Grujić et al., 2015,Ahlawat et al., 2010). So the highest contribution of SMC is optimal for *Trichoderma* spp. abundance, which covers our second research question, however more treatments for SMC are needed to be investigated in the future to support the result since G5 treatment was lost from the beginning of the experiment.

The plant growth-promoting rhizobacteria (PGPR) such as (*Agrobacterium radiobacter*, *Bacillus licheniformis*, and *Pseudomonas* spp) have indicated antagonistic effects against disease rate caused by both *P. cactorum* (68%) and *P. fragariae* (40%) in strawberry plants after four weeks in soil artificially inoculated with the pathogens. (Koch et al., 1998). The results of the present study showed a strong positive correlation between both of *Trichoderma* spp. and *Pseudomonas* spp and the macronutrients N, P and K and some of the micronutrients like Cu and Cl in the microbiota, besides releasing some metabolic enzymes like chitinase from *Trichoderma* spp. to parasite selective pathogens (St. Martin C.C.G. 2015), that could help in strawberry cultivation systems by addressing the antagonistic microbial benefits against pathogens and the utilization of nutrients availability in the root zone.

It has been pointed out by Stewart et al. (1998b) that SMC affects the availability of inorganic N concentration in the soil by increasing the growth and the yield.

The enzyme activities showed positive common results of the indigenous rhizosphere microbes in the treatments reported earlier by (Trejo-Hernandez et al. 2001), which reflect a high ability to release more carbohydrates, organic phosphorus, nutrients, and amino acids. Those compounds are vital for strawberry growth and plant development.

The dry matter reflected information about the biotic and abiotic conditions during the strawberry plant growth stage, which contributes to optimizing the production system by allocating resources for the fruiting and yield (Larson and Shaw, 1996). Dry matter was highest in treatment G3, which indicates why this treatment was significant in growth.

Analysis of SMC at the beginning of the experiment showed a carbon to nitrogen ratio (C:N) of 21:1, since the optimal C:N ratio of growing media is 20:1–40:1(Abad et al. 1989) high levels of C:N could negatively affect the growth due to the microorganisms competition with the strawberry plants for the availability of N in the root area. The C:N ratio of organic matter decreased at harvest and reached 13:1 because the C consumed, and N conserved due to the microbial activity (Jackson et al. 1989; Kaye and Hart 1997; Cheng and Bledsoe 2004). The nutrient analyses at the end of the experiment showed that the abundance of macroelements was significantly higher in G2 treatment, followed by G3 and G4 treatments, respectively. Furthermore, the abundance of most of the microelements was significantly higher in G2 treatment followed by G3 and G4 treatments, respectively, reflected the relationship between the amount of SMC in the growing media and the nutrient availably in the root area. The more SMC added to the growing media, the higher for nutrient content, which covers our third research question.

Strawberry growers could thereby minimize the extra charges of artificial fertilizers and pesticides in the conventional strawberry cultivation systems or the extra expenses of adding organic pesticides or fertilizers in the organic systems.

6. Conclusion

It could be recommended to adjust the alkalinity by adding some acids or using the phosphoric fertilizers at the planting stage to strengthen the root system. In addition to that, calibrating and adjusting the pH if SMC is used in soil preparation.

SMC could supply and contribute to the nutriment of strawberry plants in the treatments although SMC contains high rates of the nutrients that generally raise the salinity and negatively affects the plant growth.

The findings showed that G3 is optimal with respect to the growth parameters and G4 with respect to the abundance of microorganisms, these two treatments could be the optimal proposal to be implemented for addressing the SMC in growing media preparation. Losing the G5 samples is still suspicious in the presence of 100% SMC if we suspect in high salinity and pH and it needs more investigations and focusing on the characteristics of the physical mixture especially the porosity if changes to be heavier and difficult for nutrient absorptions according to the peat contribution with the SMC. So, it could be recommended G3 that the optimal treatment for both the growth and the abundance of microorganisms.

The microorganism's enzyme activities are potentially useful for the bioremediation and biodegradation of pollutants and other industrial biotechnology purposes releasing many important elements for the growth of strawberry plants as reported by Phan and Sabaratnam, (2012) and García-Delgado et al., (2015).

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8. Appendix

Appendix 1: M9 minimal media agar medium preparation

1-M9 minimal salts solution (5X concentrate):

To 800 mL of distilled/deionized water add the followings:

64g $Na_2HPO_4.7H_2O$

15g KH_2PO_4

2.5g NaCl

5.0g NH_4Cl

Sterilize by autoclaving (or filter sterilization if autoclave is not available.)

2. Preparation of (1 M $MgSO_4$):

To 100 mL distilled/deionized water we add the followings:

24.65 g $MgSO_4.7H_2O$

3. Preparation of 40% glucose (w/v):

To 100 ml distilled/deionized water add the followings:

40g Glucose

Note: Adding glucose to stirring water in a beaker and never attempt to add water to glucose.

4. Preparation of (1M $CaCl_2$):

To 100 ml distilled/deionized water add the followings:

147.014g $CaCl_2 \cdot 2dH_2O$

Method for preparation of (1L of media) minimal medium:

200 ml 5X M9 salts solution

800ml of distilled water

15g of agar media if agar plates are to be poured.

After autoclaving, swirl to mix evenly at room temperature (until you can place your hand on the flask for 2 seconds) then add:

2ml of 1M $MgSO_4$ solution

0.1ml of 1M $CaCl_2$ solution

20 ml of 20% glucose.

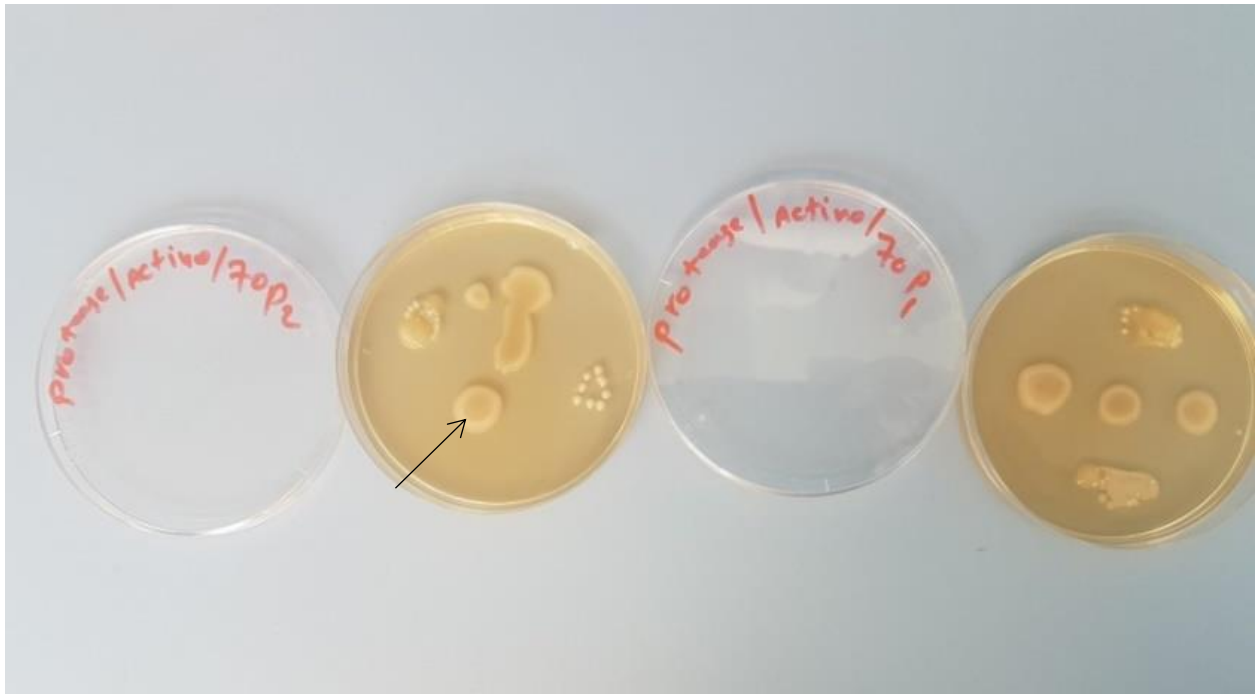
Appendix 2: The result of enzyme activities in different media protease, amylase, and organic P for all pure culture in selective media using two replicates 1 and 2 of each treatment 100% Peat, 100% SMC, 70 % Peat:30% SMC, and 50% Peat:50% SMC.

Enzyme	Medium	Replicates	Result	Enzyme	Medium	Replicates	Result	Enzyme	Medium	Replicates	Result
Protease	KB	100%P1	+	Amylase	KB	100%P1	+	Organic P	KB	100%P1	+
Protease	KB	100%P2	+	Amylase	KB	100%P2	+	Organic P	KB	100%P2	+
Protease	KB	100%SMC1	+	Amylase	KB	100%SMC1	+	Organic P	KB	100%SMC1	+
Protease	KB	100%SMC2	+	Amylase	KB	100%SMC2	+	Organic P	KB	100%SMC2	+
Protease	KB	70% P:30% SMC 1	+	Amylase	KB	70% P:30% SMC 1	+	Organic P	KB	70% P:30% SMC 1	+
Protease	KB	70% P:30% SMC 2	+	Amylase	KB	70% P:30% SMC 2	+	Organic P	KB	70% P:30% SMC 2	+
Protease	KB	50% P:50% SMC 1	+	Amylase	KB	50% P:50% SMC 1	+	Organic P	KB	50% P:50% SMC 1	+
Protease	KB	50% P:50% SMC 2	+	Amylase	KB	50% P:50% SMC 2	+	Organic P	KB	50% P:50% SMC 2	+
Protease	TSA	100%P1	+	Amylase	TSA	100%P1	+	Organic P	TSA	100%P1	+
Protease	TSA	100%P2	+	Amylase	TSA	100%P2	+	Organic P	TSA	100%P2	+
Protease	TSA	100%SMC1	+	Amylase	TSA	100%SMC1	+	Organic P	TSA	100%SMC1	+
Protease	TSA	100%SMC2	+	Amylase	TSA	100%SMC2	+	Organic P	TSA	100%SMC2	+
Protease	TSA	70% P:30% SMC 1	+	Amylase	TSA	70% P:30% SMC 1	+	Organic P	TSA	70% P:30% SMC 1	+
Protease	TSA	70% P:30% SMC 2	+	Amylase	TSA	70% P:30% SMC 2	+	Organic P	TSA	70% P:30% SMC 2	+
Protease	TSA	50% P:50% SMC 1	+	Amylase	TSA	50% P:50% SMC 1	+	Organic P	TSA	50% P:50% SMC 1	+
Protease	TSA	50% P:50% SMC 2	+	Amylase	TSA	50% P:50% SMC 2	+	Organic P	TSA	50% P:50% SMC 2	+
Protease	TSM	100%P1	+	Amylase	TSM	100%P1	+	Organic P	TSM	100%P1	+
Protease	TSM	100%P2	+	Amylase	TSM	100%P2	+	Organic P	TSM	100%P2	+

Protease	TSM	100%SMC1	+	Amylase	TSM	100%SMC1	+	Organic P	TSM	100%SMC1	+
Protease	TSM	100%SMC2	+	Amylase	TSM	100%SMC2	+	Organic P	TSM	100%SMC2	+
Protease	TSM	70% P:30% SMC 1	+	Amylase	TSM	70% P:30% SMC 1	+	Organic P	TSM	70% P:30% SMC 1	+
Protease	TSM	70% P:30% SMC 2	+	Amylase	TSM	70% P:30% SMC 2	+	Organic P	TSM	70% P:30% SMC 2	+
Protease	TSM	50% P:50% SMC 1	+	Amylase	TSM	50% P:50% SMC 1	+	Organic P	TSM	50% P:50% SMC 1	+
Protease	TSM	50% P:50% SMC 2	+	Amylase	TSM	50% P:50% SMC 2	+	Organic P	TSM	50% P:50% SMC 2	+
Protease	N.A	100%P1	+	Amylase	N.A	100%P1	+	Organic P	N.A	100%P1	+
Protease	N.A	100%P2	+	Amylase	N.A	100%P2	+	Organic P	N.A	100%P2	+
Protease	N.A	100%SMC1	+	Amylase	N.A	100%SMC1	+	Organic P	N.A	100%SMC1	+
Protease	N.A	100%SMC2	+	Amylase	N.A	100%SMC2	+	Organic P	N.A	100%SMC2	+
Protease	N.A	70% P:30% SMC 1	+	Amylase	N.A	70% P:30% SMC 1	+	Organic P	N.A	70% P:30% SMC 1	+
Protease	N.A	70% P:30% SMC 2	+	Amylase	N.A	70% P:30% SMC 2	+	Organic P	N.A	70% P:30% SMC 2	+
Protease	N.A	50% P:50% SMC 1	+	Amylase	N.A	50% P:50% SMC 1	+	Organic P	N.A	50% P:50% SMC 1	+
Protease	N.A	50% P:50% SMC 2	+	Amylase	N.A	50% P:50% SMC 2	+	Organic P	N.A	50% P:50% SMC 2	+
Protease	MB	100%P1	+	Amylase	MB	100%P1	+	Organic P	MB	100%P1	+
Protease	MB	100%P2	+	Amylase	MB	100%P2	+	Organic P	MB	100%P2	+
Protease	MB	100%SMC1	+	Amylase	MB	100%SMC1	+	Organic P	MB	100%SMC1	+
Protease	MB	100%SMC2	+	Amylase	MB	100%SMC2	+	Organic P	MB	100%SMC2	+
Protease	MB	70% P:30% SMC 1	+	Amylase	MB	70% P:30% SMC 1	+	Organic P	MB	70% P:30% SMC 1	+
Protease	MB	70% P:30% SMC 2	+	Amylase	MB	70% P:30% SMC 2	+	Organic P	MB	70% P:30% SMC 2	+
Protease	MB	50% P:50% SMC 1	+	Amylase	MB	50% P:50% SMC 1	+	Organic P	MB	50% P:50% SMC 1	+

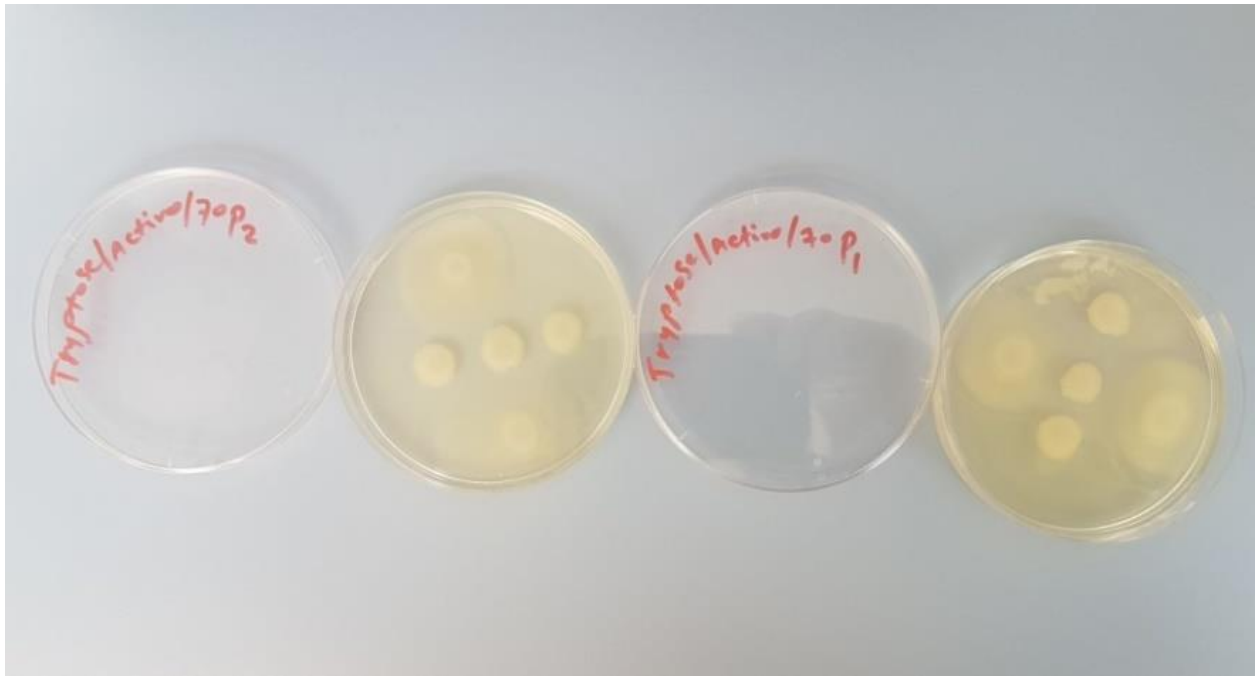
Protease	MB	50% P:50% SMC 2	+	Amylase	MB	50% P:50% SMC 2	+	Organic P	MB	50% P:50% SMC 2	+
Protease	MA	100%P1	+	Amylase	MA	100%P1	+	Organic P	MA	100%P1	+
Protease	MA	100%P2	+	Amylase	MA	100%P2	+	Organic P	MA	100%P2	+
Protease	MA	100%SMC1	+	Amylase	MA	100%SMC1	+	Organic P	MA	100%SMC1	+
Protease	MA	100%SMC2	+	Amylase	MA	100%SMC2	+	Organic P	MA	100%SMC2	+
Protease	MA	70% P:30% SMC 1	+	Amylase	MA	70% P:30% SMC 1	+	Organic P	MA	70% P:30% SMC 1	+
Protease	MA	70% P:30% SMC 2	+	Amylase	MA	70% P:30% SMC 2	+	Organic P	MA	70% P:30% SMC 2	+
Protease	MA	50% P:50% SMC 1	+	Amylase	MA	50% P:50% SMC 1	+	Organic P	MA	50% P:50% SMC 1	+
Protease	MA	50% P:50% SMC 2	+	Amylase	MA	50% P:50% SMC 2	+	Organic P	MA	50% P:50% SMC 2	+

Appendix 3: The enzyme activity of *Actinomyces* spp. in the Protease medium from the G3 microbiota shows the zone of clearance around the colony.


protease/Active/70P2
protease/Active/70P1

Appendix 4: The enzyme activity of *Bacillus* spp. in the amylase medium from the G3 microbiota.

Appendix 5: The enzyme activity of *Actinomyces* spp. in the amylase medium from the G3 microbiota.



Appendix 6: The relationship between the nutrients, growth parameters and the abundance of microorganisms in the microbiota of strawberry

		pH	EC mS/cm	N mg/l	Nitrate mg/l	Ammonium mg/l	P mg/l	K mg/l	Mg mg/l	S mg/l	Ca mg/l	Mn mg/l	B mg/l	Cu mg/l	Fe mg/l	Zn mg/l	Mo mg/l	Na mg/l	Cl mg/l	Al mg/l	KB Log$_{10}$CFU/g
pH	Pearson Correlation		.197	.125	.133	-.329	.055	.045	.199	-.293	.147	-.101	.078	.000	.	.062	-.125	.022	-.061	.085	.379
	Sig. (2-tailed)		.393	.589	.565	.145	.813	.847	.387	.197	.525	.662	.737	1.000	.	.788	.589	.926	.793	.713	.091
	N	1	1	21	21	21	21	21	1	21	1	21	21	21	1	1	21	1	21	1	21
EC mS/cm	Pearson Correlation	.197		.859**	.852**	.740**	.782**	.941*	.463*	.985**	.981**	.462*	.862**	.813**	.	.971**	-.194	.938**	.942**	.683**	.629**
	Sig. (2-tailed)	.393		.000	.000	.000	.000	.000	.035	.000	.000	.035	.000	.000	.	.000	.399	.000	.000	.001	.002
	N	1	1	21	21	21	21	21	1	21	1	21	21	21	1	1	21	1	21	1	21
N mg/l	Pearson Correlation	.125	.859**	1	.000**	.619**	.863**	.969*	.425	.776**	.833**	.305	.898**	.654**	.	.897**	-.277	.970**	.953**	.647**	.819**
	Sig. (2-tailed)	.589	.000		.000	.003	.000	.000	.055	.000	.000	.179	.000	.001	.	.000	.225	.000	.000	.002	.000
	N	1	1	21	21	21	21	21	1	21	1	21	21	21	1	1	21	1	21	1	21
Nitrate mg/l	Pearson Correlation	.133	.852**	1.000**	1	.606**	.865**	.965*	.424	.768**	.829**	.303	.895**	.655**	.	.895**	-.279	.966**	.946**	.647**	.826**
	Sig. (2-tailed)	.565	.000	.000		.004	.000	.000	.055	.000	.000	.182	.000	.001	.	.000	.221	.000	.000	.002	.000
	N	1	1	21	21	21	21	21	1	21	1	21	21	21	1	1	21	1	21	1	21
Ammonium mg/l	Pearson Correlation	.329	.740**	.619**	.606**	1	.510*	.702*	.278	.708**	.661**	.142	.752**	.383	.	.712**	-.132	.717**	.745**	.430	.368
	Sig. (2-tailed)	.145	.000	.003	.004		.018	.000	.223	.000	.001	.539	.000	.086	.	.000	.570	.000	.000	.052	.101
	N	1	1	21	21	21	21	21	1	21	1	21	21	21	1	1	21	1	21	1	21
P mg/l	Pearson Correlation	.055	.782**	.863**	.865**	.510*	1	.866*	.371	.722**	.821**	.382	.869**	.692**	.	.864**	-.300	.863**	.793**	.607**	.863**

	Sig. (2-tailed)	813		000		.000	000		.018			.000	097		.000	000		.088	.000		.001			000		.186	000		.000	004		.000
	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
	Pearson Correlation	045		941**		.969**	965**		.702**		.866**	1	457*		.882**	920**		.395	.935**		.746**	a		955**		-.267	997**		.981**	.691**		.796**
	Sig. (2-tailed)	847		000		.000	000		.000		.000		037		.000	000		.076	.000		.000			000		.242	000		.000	001		.000
K mg/l	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
	Pearson Correlation	.199		463*		.425	424		.278		.371	.457*			.420	410		.216	.512*		.453*	a		455*		-.178	460*		.476*	.567**		.460*
	Sig. (2-tailed)	387		035		.055	055		.223		.097	.037			.058	065		.347	.018		.039			038		.439	036		.029	007		.036
Mg mg/l	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
	Pearson Correlation	.293		985**		.776**	768**		.708**		.722**	*	.882*	420		1	973**		.493*	.779**		.808**	a		930**		-.168	876**		.891**	.631**		.527*
	Sig. (2-tailed)	197		000		.000	000		.000		.000	.000	058			000		.023	.000		.000			000		.468	000		.000	002		.014
S mg/l	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
	Pearson Correlation	.147		981**		.833**	829**		.661**		.821**	*	.920*	410		.973**		1	.526*	.839**		.868**	a		972**		-.222	913**		.893**	.676**		.669**
	Sig. (2-tailed)	525		000		.000	000		.001		.000	.000	065			000		.014	.000		.000			000		.333	000		.000	001		.001
Ca mg/l	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
	Pearson Correlation	.101		462*		.305	303		.142		.382	.395	216		.493*	526*		1	.290		.531*	a		432		.385	388		.373	.354		.322
	Sig. (2-tailed)	662		035		.179	182		.539		.088	.076	347		.023	014			.202		.013			051		.085	082		.096	115		.155
Mn mg/l	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
	Pearson Correlation	078		862**		.898**	895**		.752**		.869**	*	.935*	512*		.779**	839**		.290	1		.681**	a		917**		-.296	941**		.894**	.704**		.859**
	Sig. (2-tailed)	737		000		.000	000		.000		.000	.000	018		.000	000		.202			.001			000		.193	000		.000	000		.000
B mg/l	N	1		1		21	1	2	21		21	21	1	2	21	1	2	21	21		21	1	2	1	2	21	1	2	21	1	2	21
Cu mg/l	Pearson					.654**			.383		.692**		.746*			.808**		.531*	.681**		1					-.218			.671**			.658**

	Correlation	000	813**		655**			*	453*		868**				a	843**		717**		.745**	
	Sig. (2-tailed)	.000	000	.001	001	.086	.001	.000	039	.000	000	.013	.001		.	000	.341	000	.001	000	.001
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Fe mg/l	Pearson Correlation	a	a	.a	a	.a	.a	.a	a	.a	a	.a	.a	.a	a	a	.a	a	.a		.a	.a
	Sig. (2-tailed)			.		.	.	.		.		.	.	.			.		.		.	.
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Zn mg/l	Pearson Correlation	.062	971**	.897**	895**	.712**	.864**	.955*	455*	.930**	972**	.432	.917**	.843**	a		1	-.232	951**	.924**	.735**	.749**
	Sig. (2-tailed)	788	000	.000	000	.000	.000	.000	038	.000	000	.051	.000	.000			.312	000	.000	000	.000	
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Mo mg/l	Pearson Correlation	.125	.194	-.277	.279	-.132	-.300	-.267	.178	-.168	.222	.385	-.296	-.218	a	.232	1	.253	-.218	235	-.350	
	Sig. (2-tailed)	589	399	.225	221	.570	.186	.242	439	.468	333	.085	.193	.341		312		268	.342	306	.120	
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Na mg/l	Pearson Correlation	022	938**	.970**	966**	.717**	.863**	.997*	460*	.876**	913**	.388	.941**	.717**	a	951**	-.253	1	.984**	.681**	.795**	
	Sig. (2-tailed)	926	000	.000	000	.000	.000	.000	036	.000	000	.082	.000	.000		000	.268		.000	001	.000	
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Cl mg/l	Pearson Correlation	.061	942**	.953**	946**	.745**	.793**	.981*	476*	.891**	893**	.373	.894**	.671**	a	924**	-.218	984**	1	.673**	.697**	
	Sig. (2-tailed)	793	000	.000	000	.000	.000	.000	029	.000	000	.096	.000	.001		000	.342	000		001	.000	
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Al mg/l	Pearson Correlation	.085	.683**	-.647**	.647**	-.430	-.607**	.691**	.567**	-.631**	.676**	-.354	-.704**	-.745**	a	.735**	.235	.681**	-.673**	1	-.646**	
	Sig. (2-tailed)	713	001	.002	002	.052	.004	.001	007	.002	001	.115	.000	.000		000	.306	001	.001		.002	
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21

	Pearson Correlation	379		629**	.819**	826**		.368	.863**		.796*	460*		.527*	669**		.322	.859**	.658**	a		749**		-.350	795**		.697**	.646**		1
	Sig. (2-tailed)	091		002	.000	000		.101	.000		.000	036		.014	001		.155	.000	.001			000		.120	000		.000	002		
KB Log10 CFU/g	N	1		1	21	1		21	21		21	1		21	1		21	21	21	1		1		21	1		21	1		21
	Pearson Correlation	256		.575**	-.509*	.508*		-.492*	-.621**		.551**	.490*		-.554**	.573**		-.327	-.655**	-.516*	a		.600**		.201	.566**		-.551**	759**		-.591**
	Sig. (2-tailed)	263		006	.019	019		.024	.003		.010	024		.009	007		.149	.001	.017			004		.383	007		.010	000		.005
MA Log10 CFU/g	N	1		1	21	1		21	21		21	1		21	1		21	21	21	1		1		21	1		21	1		21
	Pearson Correlation	.263		.367	-.528*	.534*		-.247	-.625**		.508*	.373		-.296	.412		.084	-.613**	-.432	a		.477*		.523*	.514*		-.425	505*		-.757**
	Sig. (2-tailed)	250		102	.014	013		.280	.002		.019	096		.192	064		.716	.003	.051			029		.015	017		.055	019		.000
Bacillus/N.A Log10 CFU/g	N	1		1	21	1		21	21		21	1		21	1		21	21	21	1		1		21	1		21	1		21
	Pearson Correlation	.276		.569**	-.722**	.727**		-.369	-.801**		.712**	.443*		-.474*	.611**		-.209	-.783**	-.583**	a		.663**		.455*	.705**		-.618**	614**		-.904**
	Sig. (2-tailed)	225		007	.000	000		.099	.000		.000	044		.030	003		.363	.000	.006			001		.038	000		.003	003		.000
TSA Log10 CFU/g	N	1		1	21	1		21	21		21	1		21	1		21	21	21	1		1		21	1		21	1		21
	Pearson Correlation	038		652**	.723**	714**		.556**	.603**		.742*	387		.622**	605**		.148	.694**	.491*	a		641**		-.192	743**		.740**	.364		.590**
	Sig. (2-tailed)	869		001	.000	000		.009	.004		.000	083		.003	004		.523	.000	.024			002		.405	000		.000	105		.005
TSM Log10 CFU/g	N	1		1	21	1		21	21		21	1		21	1		21	21	21	1		1		21	1		21	1		21
	Pearson Correlation	102		.393	-.194	.187		-.110	-.016		-.260	030		-.445*	.379		-.126	-.020	-.335	a		.302		.001	.230		-.285	.022		.185
	Sig. (2-tailed)	660		078	.400	417		.635	.945		.254	896		.043	090		.585	.930	.138			183		.995	316		.210	923		.422
MB Log10 CFU/g	N	1		1	21	1		21	21		21	1		21	1		21	21	21	1		1		21	1		21	1		21
	Pearson Correlation	411		.589**	-.432	.433*		-.365	-.276		.444*	.398		-.601**	.570**		-.160	-.341	-.532*	a		.526*		.335	.437*		-.477*	337		-.184
	Sig. (2-tailed)	064		005	.050	050		.104	.226		.044	074		.004	007		.488	.130	.013			014		.138	047		.029	135		.423
Number of leaves	N				21			21	21		21			21			21	21	21					21			21			21

		1	1		1				1		1				1	1		1		1	
	Pearson Correlation	.328	.666**	-.783**	.789**	-.345	-.747**	.768**	.320	-.573**	.684**	-.148	-.745**	-.637**	a	.769**	.328	.759**	-.686**	.528*	-.764**
	Sig. (2-tailed)	.147	.001	.000	.000	.125	.000	.000	.157	.007	.001	.523	.000	.002		.000	.147	.000	.001	.014	.000
Plant Height CM	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	Pearson Correlation	.393	.475*	-.551**	.554**	-.179	-.553**	.558**	.229	-.426	.495*	-.406	-.550**	-.489*	a	.569**	-.040	.551**	-.490*	.366	-.593**
	Sig. (2-tailed)	.078	.029	.010	.009	.438	.009	.009	.318	.054	.022	.068	.010	.025		.007	.864	.010	.024	.103	.005
Petiole length CM	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21

		MA Log_{10} CFU/g	Bacillus/N.A Log_{10} CFU/g	TSA Log_{10} CFU/g	TSM Log_{10} CFU/g	MB Log_{10} CFU/g	Number of leaves	Plant Height CM	Petiole length CM	
	Pearson Correlation	.256	-.263	-.276	.038	.102	.411	-.328	-.393	
	Sig. (2-tailed)	.263	.250	.225	.869	.660	.064	.147	.078	
pH	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.575**	-.367	-.569**	.652**	-.393	-.589**	-.666**	-.475*	
	Sig. (2-tailed)	.006	.102	.007	.001	.078	.005	.001	.029	
EC mS/cm	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.509*	-.528*	-.722**	.723**	-.194	-.432	-.783**	-.551**	
	Sig. (2-tailed)	.019	.014	.000	.000	.400	.050	.000	.010	
N mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.508*	-.534*	-.727**	.714**	-.187	-.433*	-.789**	-.554**	
	Sig. (2-tailed)	.019	.013	.000	.000	.417	.050	.000	.009	
Nitrate mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.492*	-.247	-.369	.556**	-.110	-.365	-.345	-.179	
	Sig. (2-tailed)	.024	.280	.099	.009	.635	.104	.125	.438	
Ammonium mg/l	N	21	21	21	21	21	21	21	21	

	Pearson Correlation	-.621**	-.625**	-.801**	.603**	-.016	-.276	-.747**	-.553**	
	Sig. (2-tailed)	.003	.002	.000	.004	.945	.226	.000	.009	
P mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.551**	-.508*	-.712**	.742**	-.260	-.444*	-.768**	-.558**	
	Sig. (2-tailed)	.010	.019	.000	.000	.254	.044	.000	.009	
K mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.490*	-.373	-.443*	.387	.030	-.398	-.320	-.229	
	Sig. (2-tailed)	.024	.096	.044	.083	.896	.074	.157	.318	
Mg mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.554**	-.296	-.474*	.622**	-.445*	-.601**	-.573**	-.426	
	Sig. (2-tailed)	.009	.192	.030	.003	.043	.004	.007	.054	
S mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.573**	-.412	-.611**	.605**	-.379	-.570**	-.684**	-.495*	
	Sig. (2-tailed)	.007	.064	.003	.004	.090	.007	.001	.022	
Ca mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.327	.084	-.209	.148	-.126	-.160	-.148	-.406	
	Sig. (2-tailed)	.149	.716	.363	.523	.585	.488	.523	.068	
Mn mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.655**	-.613**	-.783**	.694**	-.020	-.341	-.745**	-.550**	
	Sig. (2-tailed)	.001	.003	.000	.000	.930	.130	.000	.010	
B mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.516*	-.432	-.583**	.491*	-.335	-.532*	-.637**	-.489*	
	Sig. (2-tailed)	.017	.051	.006	.024	.138	.013	.002	.025	
Cu mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.a	.a	.a	.a	.a	.a	.a	.a	
Fe mg/l	Sig. (2-tailed)	.	.	.	.	.	.	.	.	

	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.600**	-.477*	-.663**	.641**	-.302	-.526*	-.769**	-.569**	
	Sig. (2-tailed)	.004	.029	.001	.002	.183	.014	.000	.007	
Zn mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.201	.523*	.455*	-.192	.001	.335	.328	-.040	
	Sig. (2-tailed)	.383	.015	.038	.405	.995	.138	.147	.864	
Mo mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.566**	-.514*	-.705**	.743**	-.230	-.437*	-.759**	-.551**	
	Sig. (2-tailed)	.007	.017	.000	.000	.316	.047	.000	.010	
Na mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.551**	-.425	-.618**	.740**	-.285	-.477*	-.686**	-.490*	
	Sig. (2-tailed)	.010	.055	.003	.000	.210	.029	.001	.024	
Cl mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.759**	.505*	.614**	-.364	-.022	.337	.528*	.366	
	Sig. (2-tailed)	.000	.019	.003	.105	.923	.135	.014	.103	
Al mg/l	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.591**	-.757**	-.904**	.590**	.185	-.184	-.764**	-.593**	
	Sig. (2-tailed)	.005	.000	.000	.005	.422	.423	.000	.005	
KB Log10 CFU/g	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	1	.530*	.615**	-.316	-.434*	.283	.249	.146	
	Sig. (2-tailed)		.014	.003	.163	.049	.214	.275	.526	
MA Log10 CFU/g	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.530*	1	.794**	-.524*	-.346	.013	.482*	.152	
	Sig. (2-tailed)	.014		.000	.015	.124	.955	.027	.512	
Bacillus/N.A Log_{10} CFU/g	N	21	21	21	21	21	21	21	21	
TSA Log_{10} CFU/g	Pearson Correlation	.615**	.794**	1	-.422	-.234	.263	.661**	.258	

	Sig. (2-tailed)	.003	.000		.057	.307	.250	.001	.259	
	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.316	-.524*	-.422	1	-.133	-.146	-.410	-.478*	
	Sig. (2-tailed)	.163	.015	.057		.565	.528	.065	.029	
TSM Log_{10} CFU/g	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	-.434*	-.346	-.234	-.133	1	.429	.321	.228	
	Sig. (2-tailed)	.049	.124	.307	.565		.052	.156	.320	
MB Log_{10} CFU/g	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.283	.013	.263	-.146	.429	1	.319	.012	
	Sig. (2-tailed)	.214	.955	.250	.528	.052		.158	.958	
Number of leaves	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.249	.482*	.661**	-.410	.321	.319	1	.658**	
	Sig. (2-tailed)	.275	.027	.001	.065	.156	.158		.001	
Plant Height CM	N	21	21	21	21	21	21	21	21	
	Pearson Correlation	.146	.152	.258	-.478*	.228	.012	.658**	1	
	Sig. (2-tailed)	.526	.512	.259	.029	.320	.958	.001		
Petiole length CM	N	21	21	21	21	21	21	21	21	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

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