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Variation in Pathogenic Organisms as Affected by Using Hydroponic Nutrient Wastewater in Horticultural Facilities

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Abstract: Among the horticulture facilities in Korea, the hydroponic cultivation method has been increasingly used. This study aimed to identify fungi and bacterial species in hydroponic cultivation and highlight the need for sterilization to recycle the discharged drainage. Samples were collected from a number of paprika, tomato, and strawberry hydroponic greenhouses. Vinyl and glass greenhouses were studied, and the drainage discharge was of two types: direct discharge and collection after discharge. In total, 24 fungal and six bacterial species were detected from 399 and 151 densities, respectively. The primary fungal and bacterial species identified were from the genera Fusarium, Pythium, and Phytophthora as well as Agrobacterium and Pseudomonas. Statistical analysis revealed that the species number and density of fungi and bacteria were higher in strawberry than in tomato or paprika. These values were higher for vinyl greenhouses than for glass greenhouses. This study on the reuse of drainage from hydroponic greenhouses may contribute to the recycling of resources and reduction in water pollutant emission; this will thus help us to implement sustainable horticulture practices.

Keywords: agriculture; drainage; facility; greenhouse; horticulture; recycle; sustainable; waste

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1. Introduction

The facility gardening industry in Korea has been referred to as the 'white revolution' and is becoming a major agricultural technology by producing high profits and adding value to various agricultural products through year-round cultivation. This industry has become crucial for farmers who face difficulties such as falling agricultural prices [1–5]. Since the 1990s, the facility horticulture industry has accounted for more than 40% of the total horticulture industry, driven by the increasing demand for productivity, high quality, and labor savings through automation [1–4,6–9]. Hydroponics is a cultivation method that utilizes a solid medium to provide crops with the necessary nutrients and moisture [1,2,4,10]. In Korea, hydroponics was introduced in 1954 and has gained prominence due to its ability to produce high-quality crops for both domestic consumption and exportation [1,2,4,11,12]. The costs associated with hydroponic cultivation are lower than those associated with traditional soil cultivation, and the former is also advantageous as it allows growing crops in different ways [1,2,4,13,14]. In addition, hydroponic cultivation is an easy method for managing temperature and humidity, pest control, disease prevention, and nutrient supply [1,2,4,15–18]. As a result, the proportion of the cultivation area has been increasing [1,2,4,13,14]. In developed European countries, such as the Netherlands, the percentage of hydroponics farms that reuse nutrient solutions is 95%, while in Japan, it is 15%. In contrast, South Korea has a much lower percentage, less than 5% [1,2,4,19–26]. The Netherlands implemented a mandate in 2004 requiring all greenhouses to be converted to circular systems. Additionally, by 2024, hydroponic farms in the Netherlands are required to adopt recycling methods to prevent the pollution of soil, nearby rivers, and groundwater caused by the discharge of wastewater from greenhouses [1,2,4,20]. The area dedicated to hydroponics in South Korea has experienced significant growth, expanding nearly fivefold from 811 hectares in 2003 to 4224 hectares in 2018 [1,2,4,15]. Greenhouses in Korea have been built up for environmental friendly facilities with a recycling and reprocessing system for the reuse of nutrients and drainage [1,2,4,24,25]. However, the majority of farmers—approximately 95%—in South Korea use non-circulating hydroponic systems with low facility costs [1,2,20,26]. This indicates an urgent requirement to establish agricultural recycling technologies to reduce the environmental pollution caused by the discharge of waste nutrients from non-circulating hydroponic systems [1,2,4,27].

Due to the growing demand for high-quality agricultural products, there has been an adoption of large-scale hydroponic systems, leading to an increasing proportion of institutional horticulture [1,2,4,28–32]. However, this trend has resulted in various environmental issues being reported, including agricultural water depletion, non-point source pollution, and loss of fertilizer components [1,2,4,28–32]. Nonetheless, hydroponics offers the advantage of a highly controllable nutrient supply, facilitating plant growth, increased quantity, and improved quality [1,2,4,33,34]. However, pathogenic microorganisms may spread rapidly within the flowing medium because of a higher chance of contact with the crop's root, which is a disadvantage of hydroponics [1,2,4,33,34]. In non-circulating hydroponics, if the nutrient solution is drained after being supplied once, and the waste nutrient solution is discharged directly into rivers, it can result in significant adverse effects, such as groundwater and soil pollution, as well as an environmental burden due to the presence of excessive nitrogen, phosphorus, pathogens, and other contaminants [1,2,20,35]. To effectively implement a circular hydroponic system, it is essential to carefully monitor and prevent the spread of pathogens, including fungi and bacteria, present in the medium and drainage, as they can cause diseases when reused. Additionally, proper treatment of the drainage volume should be conducted during reuse [1,2,4]. By employing systematic circulating hydroponic facilities, environmentally friendly cultivation techniques such as fertilizer conservation and the prevention of water pollution can be achieved [1,2,4,36].

Major bacterial pathogens found in horticulture facilities include *Agrobacterium tumefaciens*, *Erwinia carotovora* subsp. *carotovora*, and *Pseudomonas* spp. [2,4]. Such fungal pathogens include *Botryosphaeria* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp., *Phytophthora* spp., *Pythium* spp., *Leveillula taurica*, *Cladosporium* spp., and *Alternaria solani* [2,4]. In previous studies, most of the samples analyzed were collected from the crop roots and hydroponic media. The primary pathogens detected in previous studies were *Agrobacterium tumefaciens* [37,38], *Erwinia carotovora* subsp. *carotovora* [39,40], *Pseudomonas* spp. [2], *Pseudomonas syringae* [41,42], *Pseudomonas marginalis* [43–45], *Pseudomonas ciridiflava* [43–45], *Botrytis cinerea* [46], *Colletotrichum* spp. [47], *Fusarium* spp. [48–51], *Phytophthora* spp. [52–54], and *Leveillula taurica* [55,56]. Lee et al. [2] reported that fungi and bacteria detected in the media and roots moved to the drainage through the supplied nutrient solution; therefore, the present study evaluated the degree of the detection of fungi and bacteria in the drainage for each crop.

In this study, we conducted the identification, quantification, and evaluation of harmful fungal and bacterial species present in drainage water and the growing medium used for paprika, tomato, and strawberry crops, which are commonly cultivated in hydroponic greenhouses in Korea [1,2,4]. Based on the results of our analysis, we emphasize the need for effective management measures in circulating hydroponic systems to ensure the safe reuse of discharged drainage. The questions established for the study are: is sterilization necessary to reuse, and what are the main species of drainage wastewater [1,2,4]? Is there a difference in concentration from the detected species according to the type of facility and discharge type [1,2,4]? In conclusion, the main objective of this study is to provide fundamental data on the importance and viability of sterilization facilities for achieving sustainable agriculture and establishing eco-friendly horticultural facility complexes. Additionally, the study aims to contribute to the stable cultivation of circular hydroponics. By offering this valuable information, we aim to support the implementation of environmentally conscious and sustainable practices in the field of hydroponics.

2. Materials and Methods

2.1. Selection of Study Sites

The experimental plots were selected for greenhouses cultivating paprika, tomatoes, and strawberries, the most frequently grown crops in hydroponic greenhouses in South Korea [1,2,4,20,57]. Study sites were chosen for thirty-six different areas, with three each per crop (the only selection criterion was crop type), and the drainage from each site was sampled. Korea's hydroponic greenhouses usually grow from September to May [4,58–60]. In general, the crops are planted in September, ending a one-time cycle. In Korea, the maximum temperature rises to about 35 degrees from June to August, with a high humidity, making it difficult for crops to grow inside the greenhouse [5,61–63]. In some areas, crops are produced in the summer, but temperature control is difficult [64]. Therefore, the sample for the study was a place where the crops were sufficiently grown for more than 6 months (sample collection was a similar age to the crops). The samples were collected between March and May 2020, and the physicochemical properties and concentrations of nutrient solution were similar for each crop. The samples were taken once for uniformity (outside, crop age, time) in the discharge area shown in Figure 1 and Video S1.

Figure 1. Types of study crop, greenhouse, and discharged drainage.

In this study, the age and type of the medium were not considered. The reason is that all farms sterilize fungi and bacteria by performing sufficient disinfection before planting crops in September [65,66]. This study divided the horticultural facilities into vinyl and glass greenhouses and the drainage discharge types into direct discharge and collection after discharge to better understand the degree of fungal and bacterial presence in the discharged drainage according to the properties of the study site (Table 1, Figure 1, Video S1).

Table 1. Criteria for the selection of study sites.

Study Site	Type Classification
Cultivated crops (3)	Paprika (P), 12; tomato (T), 12; strawberry (S), 12
Greenhouse facility type (2)	vinyl greenhouse, 28; glass greenhouse, 8
Drainage discharge type (2)	direct discharge, 13; collection after discharge, 23

2.2. Methods for Analysis

Paprika, tomato, and strawberry, which are the most commonly grown crops in hydroponics in Korea, were selected as study crops based on the findings of the Ministry of Agriculture, Food and Rural Affairs (MAFRA) [1,2,4,6,7,9]. By analyzing the harmful fungi and bacteria most commonly found in Korean hydroponics, 57 fungi and 11 bacteria were detected [1,2,4,39,67]. The most common fungal and bacterial pathogens in Korea are *Cladosporium* spp., *Botrytis* cinerea, *Pythium* spp., *Fusarium* spp., *Colletotrichum loeosporioides*, *Pseudomonas* spp., and *Erwinia carotovora* subsp. [1,2,4,67]. These harmful fungi and bacteria are known to be typical species that require management in horticultural practices [1,2,64].

To detect the major fungal and bacterial species, DNA multi-scan analysis was performed (Eurofins Agro; Eurofins Scientific, Agro, LLC, Wageningen, The Netherlands) using the following steps [1,2,4].

First, the analysis samples were prepared (drainage samples were collected in 1 L sterile collection bottles).

Second, the sample were put into contact with a sterile liquid medium (for the release of fungal and bacterial isolates).

Third, DNA amplification was performed using the PCR process (low-level DNA detection).

Fourth, DNA was put into contact with a specific membrane.

Fifth, the visualization of attached fungi and bacteria was performed (detected pathogens were classified into six categories according to European standards: <25 colony-forming unit (CFU)/m^3 = very low (1), <100 CFU/m^3 = low (2), <500 CFU/m^3 = moderate (3), <1000 CFU/m^3 = moderate-to-high (4), <2000 CFU/m^3 = high (5), and >2000 CFU/m^3 = very high (6)).

By following these steps, researchers can identify and classify the harmful fungi and bacteria commonly found in hydroponics in Korea. This information can be used to develop appropriate management strategies to control and mitigate the effects of these pathogens in captive horticultural practices [1,2,4].

2.3. Statistical Analysis

The density of fungal and bacterial species found in the samples obtained from various cultivated crops from different target sites was analyzed using SPSS 19.0 (IBM, Armonk, NY, USA) [1,2,4]. First, the differences in the species composition and density of fungal and bacterial species based on cultivated crops were analyzed using analysis of variance (ANOVA) [1,2,4]. The differences based on the facility properties and water drainage types were analyzed using a *t*-test. The results of the analysis are presented using F- and T-values; statistical significance is marked with an asterisk (*).

3. Results

3.1. Fungi Detected in Drainage According to Crop Type

Twenty-four fungal species from four phyla, six classes, eight orders, ten families, and ten genera were observed in the thirty six hydroponic greenhouses; the observed density was 399 (Table 2, Appendices A and B), which was similarly reported in the previous study [20,68]. Approximately 3.33 species were observed at a study site with a density of approximately 11.08. Fifteen species from three phyla, four classes, six orders, seven families, and seven genera were detected in paprika among the crop types with 105 densities; in tomatoes, a density of 91 was observed for thirteen species from four phyla, five classes, five orders, seven families, and seven genera. In strawberries, we observed a density of 203 for sixteen species from three phyla, five classes, seven orders, eight families, and eight genera [68].

Table 2. Fungi detected according to the type of crop.

Division	Ph	Cl	Or	Fa	Ge	Sp.	TD
Paprika (N = 12)							
Sum	3	4	6	7	7	15	105
Average	1.08	1.25	1.50	1.75	1.75	2.83	8.8
SD	1.00	1.14	1.45	1.66	1.66	2.66	9.0
Tomato (N = 12)							
Sum	4	5	5	7	7	13	91
Average	1.25	1.25	1.25	1.33	1.33	2.50	7.58
SD	1.06	1.06	1.06	1.15	1.15	2.32	7.13
Strawberry (N = 12)							
Sum	3	5	7	8	8	16	203
Average	1.92	2.42	2.58	2.83	2.83	4.67	16.92
SD	0.51	0.79	0.90	1.11	1.11	1.72	5.78
Total (N = 36)							
Sum	4	6	8	10	10	24	399
Average	1.42	1.64	1.78	1.97	1.97	3.33	11.08
SD	0.94	1.13	1.27	1.44	1.44	2.40	8.35

Ph: phylum, Cl: class, Or: order, Fa: family, Ge: genus, Sp.: species, TD: total density, SD: standard deviation.

Clearly, the number of species and density were the highest in the case of strawberries. At one study site, the number and density were 2.83 ± 2.66 and 8.80 ± 9.00 in the case of paprika, 2.50 ± 2.32 and 7.58 ± 7.13 in tomato, and 4.67 ± 1.72 and 16.92 ± 5.78 in strawberry (Figure 2). In the hypothesis of the study, the aim was to find out whether sterilization is necessary to reuse drainage wastewater. According to the results of the fungi analysis, it was determined that sterilization was necessary to reuse drainage wastewater.

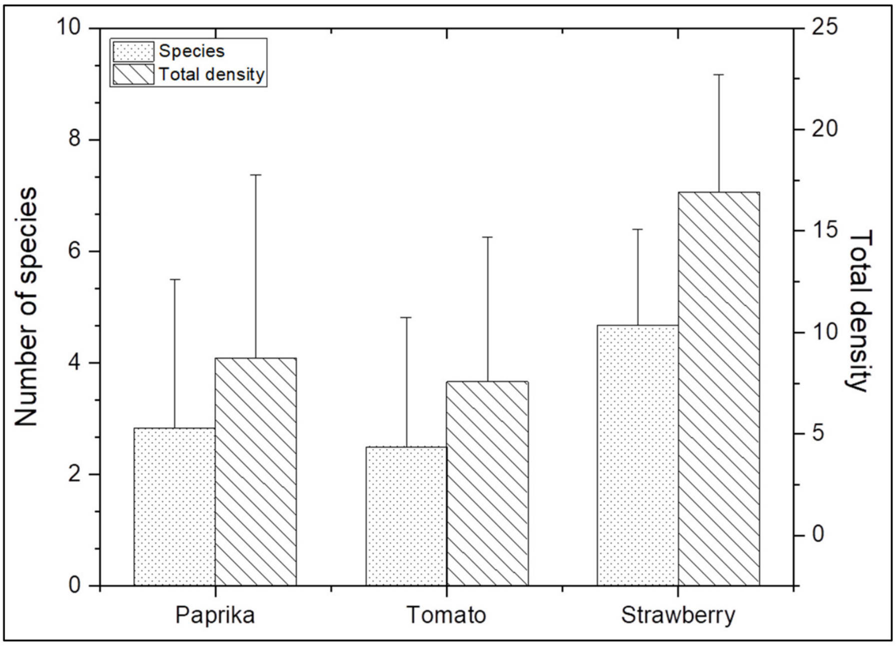

Figure 2. Average density of fungi detected at 36 study sites according to cultivated crops.

As shown in Figure 3 and Appendix B, *Alternaria* sp. was detected at one paprika, one tomato, and two strawberry sites. The mean density for the four sites was 1.50 ± 1.00.

Generally, *Alternaria* spp. infect the leaves, stems, flowers, fruits, and trees. This organism commonly causes black mold in tomatoes and peppers [68–71].

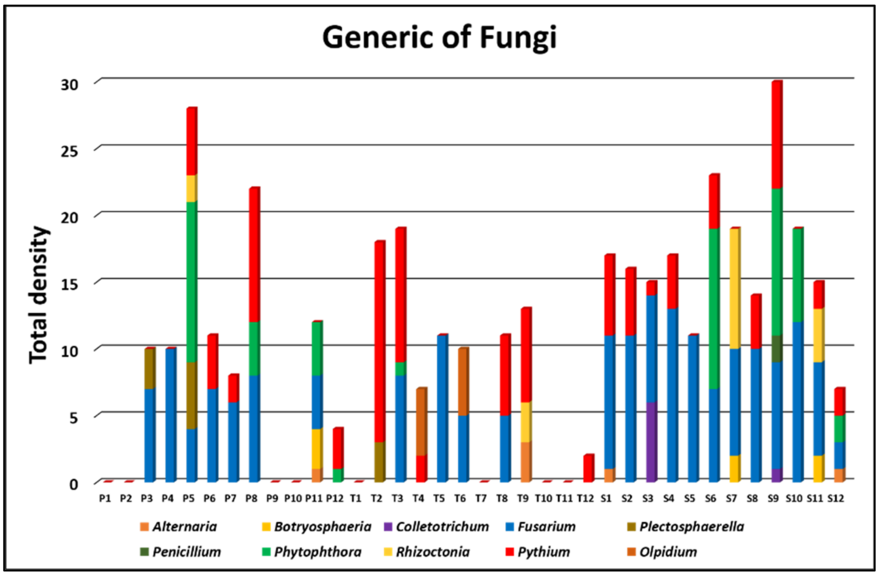

Figure 3. Total density of fungi detected at 36 study sites according to generic division; P: paprika, T: tomato, S: strawberry.

Botryosphaeria sp. was detected at the paprika and strawberry sites. The mean density for the three sites was 2.33 ± 0.47. The disease caused by *Botryosphaeria* sp., a gray fungus, is a common plant infection. *Botryosphaeria* causes Phomopsis canker of crops and kills the entire plant through the stem [72]. *Colletotrichum* sp. was found at only two strawberry sites. The mean density for the two sites was 3.50 ± 2.50. *Colletotrichum* spp. is the causal factor of anthrax that develops brown or blackish-brown spots on the leaves, turns stems or branches black, wilts them, or results in the premature falling of leaves and death [47]. This organism has been reported to be present in peppers, strawberries, sweet persimmons, apples, grapes, and blueberries [47].

Fusarium lactis was detected at two paprika sites. The mean density for the two sites was 2.50 ± 1.50. *F. oxysporum* was present at three paprika, three tomato, and 11 strawberry sites. The mean density was 3.33 ± 0.47 at three paprika sites, 3.33 ± 1.70 at three tomato sites, and 3.27 ± 1.54 at 11 strawberry sites. *F. oxysporum* f. sp. *cucum* was detected at one paprika site. The density at the site was 1. *Fusarium solani* was present at one paprika, one tomato, and four strawberry sites. The mean density for the six sites was 1.50 ± 0.50. *Fusarium* sp. was detected at seven paprika, four tomato, and 12 strawberry sites. The mean density was 4.00 ± 1.07 at seven paprika sites, 4.50 ± 1.12 at four tomato sites, and 5.42 ± 1.11 at 12 strawberry sites. *Fusarium* sp. causes root rot and wilt diseases. *F. oxysporum* is a larval fungus and an infectious soil pathogen spread by fungus gnats and causes wilt disease of tomato and lettuce. In particular, when *F. oxysporum* is spread by a fungus gnat to the medium while cultivating tomato, it causes enormous damage to the plants, withering the entire plant [48]. In addition, *F. solani* causes Fusarium wilt of soybean and rhizome rot of cucumber [49]. It was also reported to cause wilt disease in strawberry hydroponics in South Korea and other countries. The strawberry wilt disease results in malformed leaves, reduced growth, and withered seedlings. Consequently, this disease is the biggest challenge in strawberry cultivation facilities. Notably, the occurrence of wilt disease increases with the increase in the number of farms using hydroponics [50,51]. *Plectosphaerella cucumerina* was detected at one tomato and two paprika sites. The mean density for the three sites was 3.67 ± 0.94. *Penicillium* sp. was detected at one strawberry site. The density was 2. *Phytophthora cactorum* was detected at two strawberry sites. The mean density for the two sites was 5.50 ± 0.50. *P. capsici* was found at one paprika site. The density was 6. *P. cinnamomi* was detected at one paprika site. The density was 2. *P. fragariae* was detected at one paprika site and one strawberry site. The mean density for the two sites was 3.50 ± 2.50. *P. nicotianae* was detected at two strawberry sites. The mean density for the two sites was 1.50 ± 0.50. *Phytophthora* sp. was detected at three paprika, one tomato, and two strawberry sites. The mean density for the six sites was 4.17 ± 2.03. *Phytophthora* sp. is a fungus known to cause the crown rot of strawberry, which wilts leaves, rots roots, and reduces flowers. The crown rot of strawberry wilts the entire plant and occurs in leaves, branches, flower stalks, crowns, and roots. When this organism infects a plant, the infected leaves look blanched. Fungi such as *P. fragariae* and *P. nicotianae* cause the crow rot of peppers, tomatoes, and strawberries [52–54].

Rhizoctonia fragariae was found at two strawberry sites. The mean density for the two sites was 4.00 ± 2.00. *R. solani* was detected at one paprika, one tomato, and two strawberry sites. The mean density for the four sites was 2.50 ± 0.50. *Pythium aphanidermatum* was detected at three tomato and two strawberry sites. The mean density for the five sites was 2.00 ± 2.00. *P. dissotocum* was found at four paprika and four tomato sites. The mean density was 2.25 ± 1.64 at four paprika sites and 2.25 ± 1.64 at four tomato sites. *P. irregulare* was detected at one tomato site and one strawberry site. The mean density for the two sites was 2.50 ± 0.50. *Pythium* sp. was detected at five paprika, six tomato, and eight strawberry sites. The mean density for the sites was 3.00 ± 1.41 at five paprika sites, 3.17 ± 1.95 at six tomato sites, and 4.00 ± 1.50 at eight strawberry sites. *P. tracheiphyllum* was detected at two tomato sites. The mean density for the two sites was 1.50 ± 0.50. *Pythium* spp. can cause severe diseases in crops [73–75]. *Pythium* spp., a soil-borne pathogen, causes damping off and turns the color of stems and roots brown. Such lesions have been reported in ginseng cultivation [73]. *P. dissotocum* is commonly found in the hydroponic system and causes root rot [74]. *P. irregulare* is a soil-borne pathogen found in hundreds of different plant hosts and has been reported to cause root rot [75]. *P. aphanidermatum* is borne by a soil pathogen; cucumber damping-off disease is a representative disease caused by this organism. It also causes root rot and can disrupt the photosynthesis of tomatoes and cucumbers in greenhouses [73,76]. *Olpidium virulentus* was found at two tomato sites. The mean density for the two sites was 5.00 ± 0.00.

In the hypothesis of the study, the aim was to find out the main sterilization target species. The fungi present in the drainage from the horticulture facility that should be sterilized belonged to the genera *Fusarium*, *Pythium*, and *Phytophthora*.

Table 3 and Figure 4 present the data of 24 fungi detected in 36 hydroponic greenhouses according to the greenhouse and drainage types.

Table 3. Fungi detected according to the type of greenhouse and drainage.

Division	Ph	Cl	Or	Fa	Ge	Sp.	TD
Vinyl greenhouse (N = 28)							
Sum	4	6	8	10	10	24	374
Average	1.64	1.82	1.96	2.14	2.14	3.86	13.36
SD	0.87	1.02	1.14	1.33	1.33	2.32	7.83
Glass greenhouse (N = 8)							
Sum	2	3	4	5	5	8	25
Average	0.63	1.00	1.13	1.38	1.38	1.50	3.13
SD	0.74	1.31	1.55	1.77	1.77	1.77	4.39
Direct discharge (N = 13)							
Sum	3	4	5	7	7	12	141
Average	1.38	1.46	1.54	1.62	1.62	2.62	10.85
SD	0.87	0.97	1.05	1.12	1.12	1.71	7.10
Collection after discharge (N = 23)							
Sum	3	5	8	9	9	23	258
Average	1.43	1.74	1.91	2.17	2.17	3.74	11.22
SD	0.99	1.21	1.38	1.59	1.59	2.67	9.13

Ph: phylum, Cl: class, Or: order, Fa: family, Ge: genus, Sp.: species, TD: total density, SD: standard deviation.

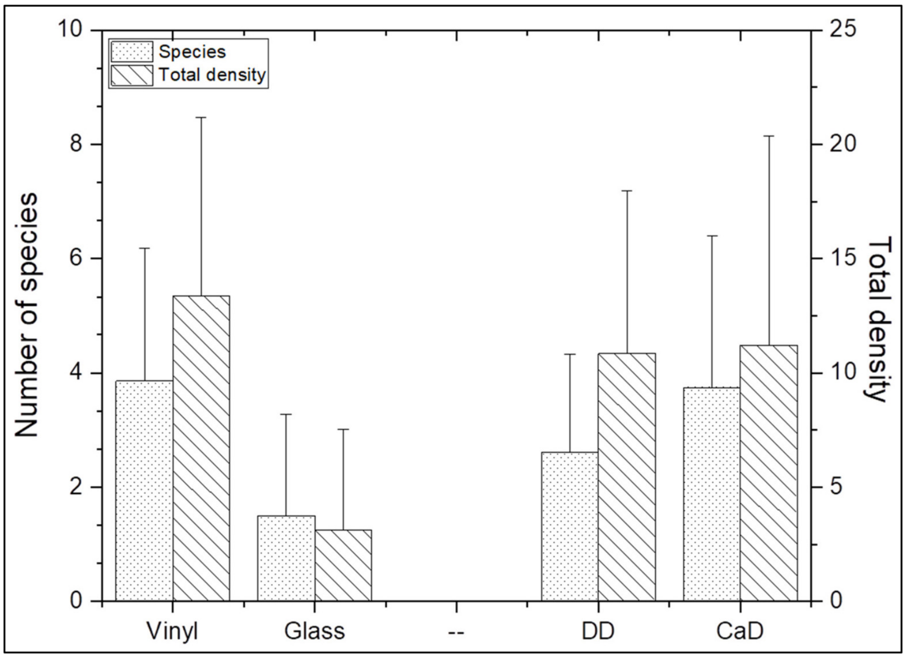

Figure 4. Average density of fungi detected according to the type of greenhouse and drainage. Vinyl: vinyl greenhouse, Glass: glass greenhouse, DD: direct discharge, CaD: collection after discharge.

The vinyl greenhouses had 3.86 ± 2.32 species and a density of 13.36 ± 7.83, whereas glass greenhouses had 1.50 ± 1.77 species and a density of 3.13 ± 4.39. Moreover, the collection after discharge sample showed 3.74 ± 2.67 species and a density of 11.22 ± 9.13, which was slightly higher than those of the direct discharge sample, which had 2.62 ± 1.71 species and a density of 10.85 ± 7.10.

The results of the detection of fungal species according to the facility type were suggested in the hypothesis of the study; the detection of species and concentrations in the vinyl greenhouses were higher than that of the glass greenhouses. This means that bacteria are being managed better in glass greenhouses. As a result of classifying the discharge type, there was no difference in the total concentration, and the CaD type was slightly higher in the number of species. Caught and collected wastewater in greenhouses can be said to be vulnerable to pathogens.

3.2. Bacteria Detected in Drainage According to Crop Type

In the 36 hydroponic greenhouses investigated in this study, six bacterial species from one phylum, two classes, three orders, three families, and three genera were detected, and the sum of the detected densities was 151 (Table 4).

On average, 1.58 ± 1.23 species were detected per site; the mean density was 4.19 ± 3.21. In total, 46 densities were detected in the case of paprika, with five species from one phylum, two classes, three orders, three families, and three genera; 28 densities were detected in tomato, with three species from one phylum, two classes, three orders, three families, and three genera. In the case of strawberries, a total of 77 densities were analyzed with five species from one phylum, two classes, two orders, two families, and two genera, showing the highest species composition.

At one study site, paprika, tomato, and strawberry showed 1.25 ± 0.87 species and 3.83 ± 2.69 densities, 0.83 ± 0.39 species and 2.33 ± 1.72 densities, and 2.67 ± 1.37 species and 6.42 ± 3.65 densities, respectively (Figure 5).

Table 4. Bacteria detected according to the type of crop.

Division	Ph	Cl	Or	Fa	Ge	Sp.	TD
Paprika (N = 12)							
Sum	1	2	3	3	3	5	46
Average	0.83	0.92	1.08	1.08	1.08	1.25	3.83
SD	0.39	0.51	0.67	0.67	0.67	0.87	2.69
Tomato (N = 12)							
Sum	1	2	3	3	3	3	28
Average	0.83	0.83	0.83	0.83	0.83	0.83	2.33
SD	0.39	0.39	0.39	0.39	0.39	0.39	1.72
Strawberry (N = 12)							
Sum	1	2	2	2	2	5	77
Average	1.00	1.58	1.58	1.58	1.58	2.67	6.42
SD	0.00	0.51	0.51	0.51	0.51	1.37	3.65
Total (N = 36)							
Sum	1	2	3	3	3	6	151
Average	0.89	1.11	1.17	1.17	1.17	1.58	4.19
SD	0.32	0.57	0.61	0.61	0.61	1.23	3.21

Ph: phylum, Cl: class, Or: order, Fa: family, Ge: genus, Sp.: species, TD: total density, SD: standard deviation.

In the hypothesis of the study, the aim was to find out whether sterilization is necessary to reuse drainage wastewater. According to the results of bacterial analysis, the necessity of sterilization to reuse drainage was recognized.

As shown in Figure 6 and Appendix B, *Agrobacterium tumefaciens* was detected at one paprika, two tomato, and seven strawberry sites. The mean density was 3.00 ± 0.00 at one paprika site, 4.00 ± 1.00 at two tomato sites, and 1.71 ± 1.39 at seven strawberry sites. *A. tumefaciens* Ti-plasmid was detected at one paprika and two strawberry sites. The mean density for the three sites was 2.00 ± 0.82. *A. tumefaciens*, the bacterium that causes crown gall, is mainly found in fruit-bearing plants such as peaches, grapes, pears, and apples [37]. It also causes crown gall to horticulture facility crops such as roses [38].

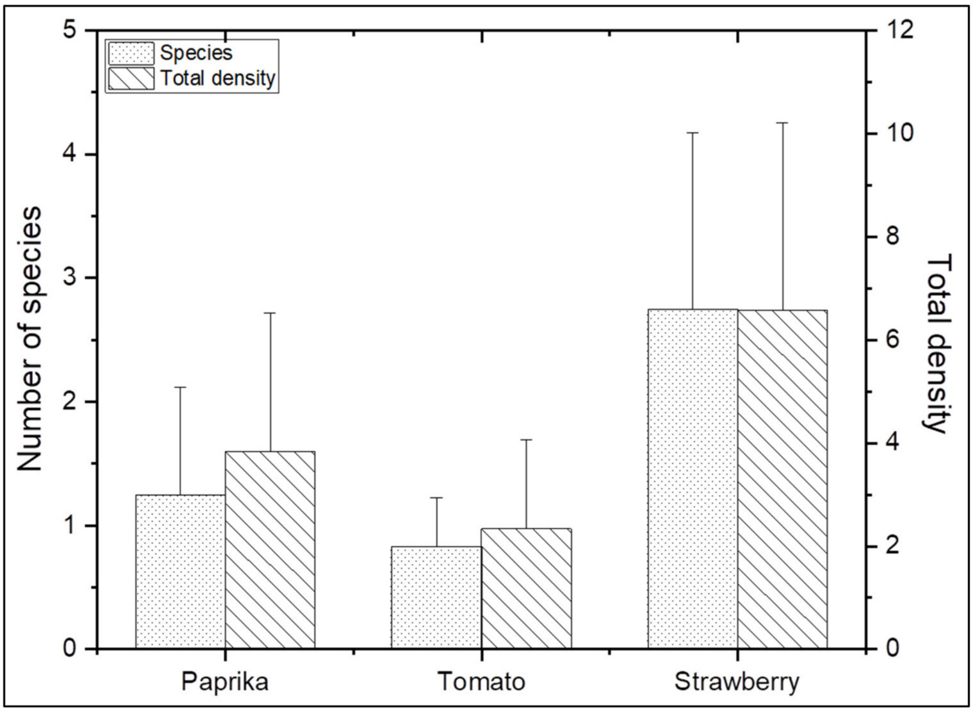

Figure 5. Average density of bacteria detected at 36 study sites according to cultivated crops.

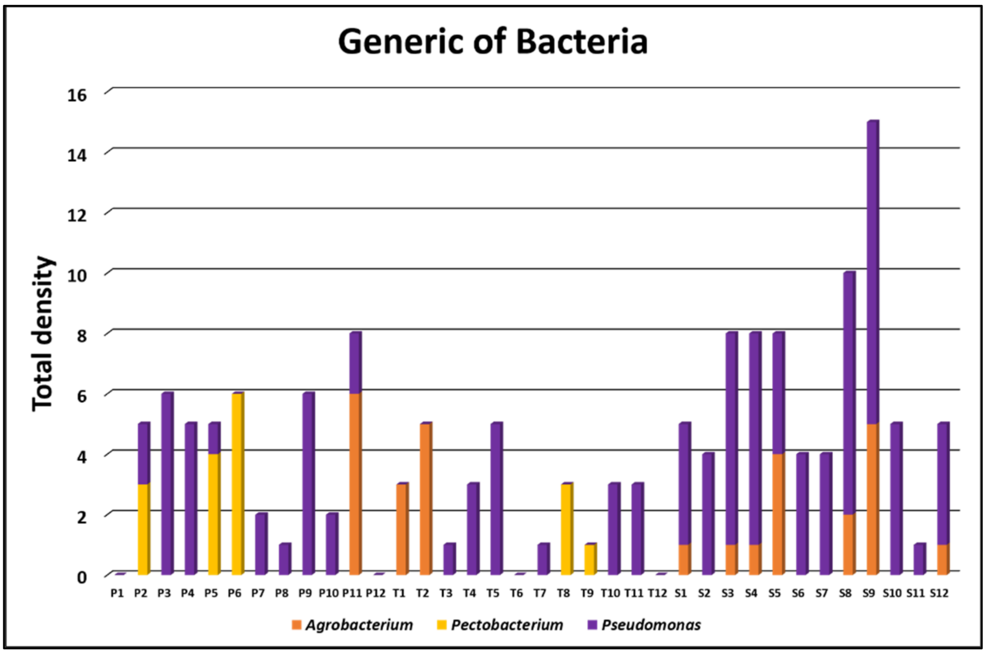

Figure 6. Total density of bacteria detected at 36 study sites according to generic division. P: paprika, T: tomato, S: strawberry.

Erwinia carotovora subsp. *carotovora* was detected in three paprika and two tomato sites. The mean density for the five sites was 3.40 ± 1.62. *E. carotovora* subsp. *carotovora* causes various diseases such as leaf spot, soft rot, Fusarium wilt, and gall of napa cabbages. When infected, spots spread to the entire plant, making it soft and rotten, resulting in a foul odor [39]. It is often found in the stems of crops in horticultural complexes [39]. In addition, it was detected in the fruit packaging unit of paprika farms, where it caused fruit rot [40].

Pseudomonas fluorescens was detected at nine paprika, six tomato, and 11 strawberry sites. The mean density was 2.89 ± 1.79 at nine paprika sites, 2.67 ± 1.37 at six tomato sites, and 3.81 ± 1.53 at 11 strawberry sites. *P. marginalis* was detected at one paprika and four strawberry sites. The mean density for the five sites was 1.00 ± 0.00. *P. viridiflava* was detected at eight strawberry sites. The mean density for the eight sites was 2.88 ± 0.78. *Pseudomonas* sp. decays plant tissues and causes blossom blight, similar to bacterial spots [38,39]. *P. marginalis* causes a red-colored phenomenon that generates small yellowish-brown and dark reddish-brown spots on ginseng and ginger, which gradually become larger and turn the entire surface brown [43]. It was also reported to cause cucumber leaf spot and bacterial blossom blight in tomatoes [44,45]. *P. fluorescens* causes infection in soil, water, and plant surfaces and is a causal agent for Fusarium wilt. It mainly acts as a soil-borne pathogen and causes Fusarium wilt in tomatoes [77]. In addition, *P. viridiflava* causes leaf necrosis, stem lesions, and basal stem and root rot in various plants. Recently, *P. viridiflava* has been reported to cause a spotting disease in the cultivation facility for Cucurbitaceae crops such as cucumbers in South Korea and other countries. This leads to a 10–20% loss in the nursery [78,79]. The spots become larger, turn leaves gray, and make the plant gradually wilt and die [78,79].

In the hypothesis of the study, the aim was to find out the main sterilization target species. The bacteria present in the drainage from the horticulture facility that should be sterilized belonged to the genera *Agrobacterium* and *Pseudomonas*.

Table 5 presents the six bacteria detected in 36 hydroponic greenhouses according to the type of greenhouse and drainage. The vinyl greenhouses had 1.21 ± 0.57 species and a density of 1.64 ± 1.25, whereas the glass greenhouse had 1.00 ± 0.76 species and a density of 1.38 ± 1.19.

Table 5. Bacteria detected according to the type of greenhouse and drainage.

Division	Ph	Cl	Or	Fa	Ge	Sp.	TD
Vinyl greenhouse (N = 28)							
Sum	1	2	3	3	3	6	124
Average	2.11	0.93	1.14	1.21	1.21	1.21	1.64
SD	0.83	0.26	0.52	0.57	0.57	0.57	1.25
Glass greenhouse (N = 8)							
Sum	1	2	2	2	2	5	27
Average	1.63	0.75	1.00	1.00	1.00	1.00	1.38
SD	0.74	0.46	0.76	0.76	0.76	0.76	1.19
Direct discharge (N = 13)							
Sum	1	2	3	3	3	6	55
Average	2.23	0.85	1.15	1.23	1.23	1.23	1.69
SD	0.73	0.38	0.69	0.73	0.73	0.73	1.32
Collection after discharge (N = 23)							
Sum	1	2	3	3	3	6	96
Average	1.87	0.91	1.09	1.13	1.13	1.13	1.52
SD	0.87	0.29	0.51	0.55	0.55	0.55	1.20

Ph: phylum, Cl: class, Or: order, Fa: family, Ge: genus, Sp.: species, TD: total density, SD: standard deviation.

Moreover, the direct discharge sample showed 1.23 ± 0.73 species and a density of 1.69 ± 1.32, which was slightly higher than those of the collection after discharge sample, which had 1.13 ± 0.55 species and a density of 1.52 ± 1.20 (Figure 7).

The results of the detection of bacterial species according to the facility type, as suggested in the hypothesis of the study, are that vinyl greenhouses and glass greenhouses were identified almost similarly. The results of classifying them into emission types were similar without any differences. Unlike fungi, it is judged that there is no difference in bacterial detection concentration according to shape or discharge type.

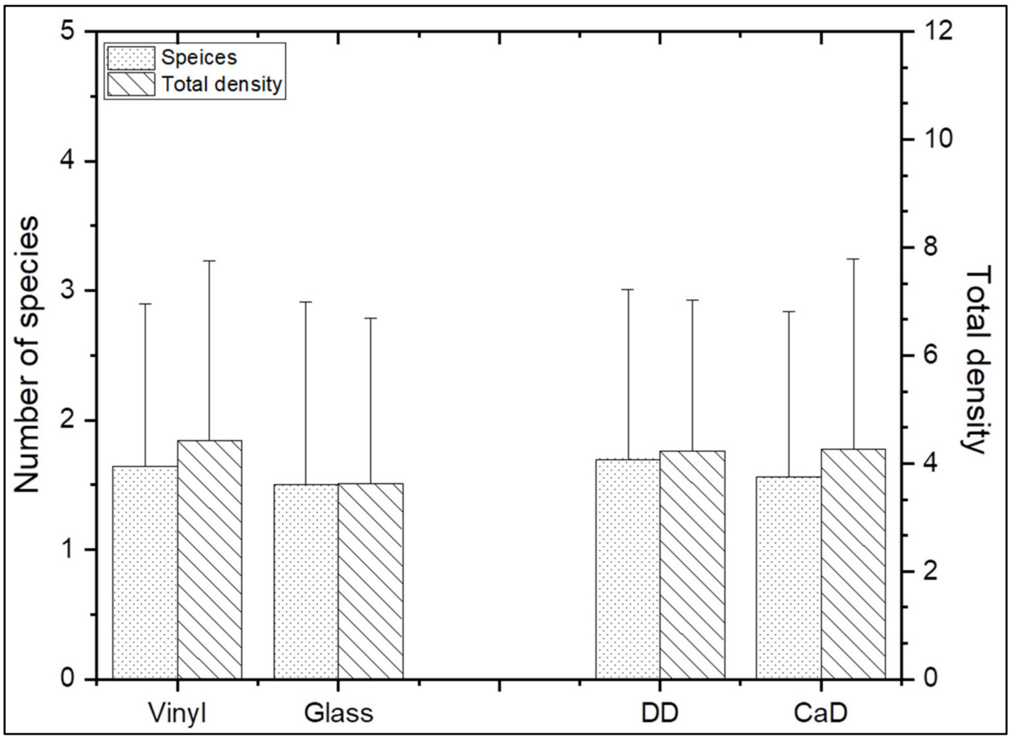

Figure 7. Average density of bacteria detected according to the type of greenhouse and drainage. Vinyl: vinyl greenhouse, Glass: glass greenhouse, DD: direct discharge, CaD: collection after discharge.

3.3. Statistical Analysis of Detected Density by Crop

This study analyzed the fungal and bacterial species composition and density detected at 12 sites among 36 sites using ANOVA (Table 6). In the case of fungi, it was significantly different at the class, order, family, and genus level with 95% confidence. Overall, strawberries showed more abundant species than tomatoes and paprika. The total density was in the order of strawberry, paprika, and tomato at a 99% confidence level.

Table 6. Results of statistical analyses according to the type of crop.

Division	Fungi		Bacteria	
	F	**PH**	**F**	**PH**
Phylum	2.952	NS	1.100	NS
Class	5.377 *	P, T < S	8.922 **	T, P < S
Order	4.504 *	T, P < S	6.079 **	T, P < S
Family	4.053 *	T < P <S	6.079 **	T, P < S
Genus	4.053 *	T < P < S	6.079 **	T, P < S
Species	3.183	NS	11.959 ***	T, P < S
Total concentration	5.627 **	T < P < S	6.517 **	T, P < S

F: F-value, PH: post hoc; test result is significant at $p = 0.05$ (*), 0.01 (**), 0.001 (***); NS: not significant, P: paprika, T: tomato, S: strawberry.

In the case of bacteria, the results of the statistical analysis were more distinct. For the sum of densities at the class, order, family, and genus levels, strawberries were significantly higher than tomatoes and paprika at a 99% confidence level. The species composition was analyzed at a 99% confidence level. Based on the statistical results, it could be determined that the density of strawberry was higher than that of tomato and that of paprika.

Greenhouses were divided into vinyl and glass greenhouses, and the difference was analyzed using the *t*-test. The results showed that the bacteria were not different between the two greenhouse types, and the species number of fungi was significantly higher in the vinyl greenhouse than in the glass greenhouse at a 95% significant level. Simultaneously, the sum of densities of the vinyl type was significantly higher (a 99% significant level) than that of the glass type (Table 7).

Table 7. Results of statistical analyses according to the type of greenhouse.

Division	Fungi		Bacteria	
	T	**PH**	**T**	**PH**
Phylum	3.003 **	G < V	1.044	NS
Class	1.887	NS	0.615	NS
Order	1.695	NS	0.874	NS
Family	1.342	NS	0.874	NS
Genus	1.342	NS	0.874	NS
Species	2.650 *	G < V	0.539	NS
Total concentration	3.518 **	G < V	0.814	NS

T: T-value, PH: post hoc; test result is significant at $p = 0.05$ (*), 0.01 (**); NS: not significant, V: vinyl, G: glass.

The glass greenhouse growth environment was better than the vinyl greenhouses in the management of temperature and humidity, crop growth, and pests. Therefore, it is assumed that these greenhouses are easier to manage. Nam [37] also confirmed the same phenomenon, showing a lower number of organisms in glass greenhouses than in vinyl greenhouses.

Water drainage was classified into the direct discharge and the collection after discharge, and the samples were analyzed using the *t*-test. In the initial stage, this study hypothesized that the density in the collection after discharge sample would be higher than that in the direct discharge sample because of a prolonged duration of stagnancy in the catchment area and the influences of water quality. The analysis results indicated that fungi and bacteria were not affected significantly by catchment (Table 8).

Table 8. Results of statistical analyses according to the type of drainage.

Division	Fungi		Bacteria	
	T	**PH**	**T**	**PH**
Phylum	−0.152	NS	−0.599	NS
Class	−0.706	NS	0.331	NS
Order	−0.848	NS	0.469	NS
Family	−1.119	NS	0.469	NS
Genus	−1.119	NS	0.469	NS
Species	−1.365	NS	0.396	NS
Total concentration	−0.126	NS	0.050	NS

T: T-value, PH: post hoc; test result is NS: not significant, DD: direct discharge, CaD: collection after discharge.

Future studies are needed to investigate the cause of the additional densities of fungi and bacteria in the drainage that were not analyzed in this study.

4. Discussion

This study found many species belonging to the genera of *Fusarium*, *Pythium*, and *Phytophthora*. The fungi present in the drainage from the horticulture facility that should be sterilized belonged to the genera *Fusarium*, *Pythium*, and *Phytophthora*. This study further suggests developing devices and technologies that can sterilize these species. Vinyl greenhouses had 3.86 ± 2.32 species and a density of 13.36 ± 7.83, whereas glass greenhouses had 1.50 ± 1.77 species and a density of 3.13 ± 4.39. The results were consistent in that glass greenhouses managed and disinfected fungi better. The direct discharge sample showed 2.62 ± 1.71 species and a density of 10.85 ± 7.10, whereas the collection after discharge sample showed 3.74 ± 2.67 species and a density of 11.22 ± 9.13, revealing that these values in the collection after discharge sample were slightly higher.

The bacteria detected included many species belonging to the genus of *Agrobacterium* and *Pseudomonas*. The bacteria present in the drainage from the horticulture facility that should be sterilized belonged to the genera *Agrobacterium* and *Pseudomonas*. This study suggests developing devices and technologies that can sterilize these species. Vinyl greenhouses had 1.21 ± 0.57 species and a density of 1.64 ± 1.25, whereas glass greenhouses had 1.00 ± 0.76 species and a density of 1.38 ± 1.19, indicating that the glass greenhouses managed and sterilized the bacteria better. The direct discharge sample showed 1.23 ± 0.73 species and a density of 1.69 ± 1.32, whereas the collection after discharge sample showed 1.13 ± 0.55 species and a density of 1.52 ± 1.20, revealing that these values in the collection after discharge sample was slightly higher.

On the basis of the results of the statistical analyses, the fungi in the case of strawberry were more abundant than those in tomato and paprika, and the combined density was in the order of strawberry, paprika, and tomato at a 99% confidence level. In the case of bacteria, it was analyzed with a 99.9% confidence level that the bacteria were more abundant in strawberry than in tomato and paprika. The density in the case of strawberry was higher than that in the cases of tomato and paprika. The species composition and the sum of densities of the fungus of vinyl greenhouses were higher than those of glass greenhouses. It can be inferred that glass greenhouses, which represent smart farming and facility modernization, have better pathogen management. However, the drainage types did not exhibit a significant difference in any of the factors studied. Future studies are warranted to identify the cause of the density between the fungi and bacteria found in the drainage. In addition, the correlation between the chemical concentration of the nutrient solution and the detection of fungi and bacteria should be investigated. This is a limitation of the study and highlights the need for further research. This study on drainage recycling will facilitate sustainable horticulture by providing insights into the recycling of resources and a reduction in water pollutants.

5. Conclusions

This study confirmed the possibility of reusing the drainage by suggesting the necessity of effectively sterilizing the drainage in horticulture nutrient solution hydroponic systems. We suggested species that need to be sterilized for water reuse. This contributes to water conservation through wastewater reuse. In addition, it contributes to carbon net-zero through the reduction and reuse of fertilizers. The results of this study are able to highlight the improvement in horticulture facilities for sustainable agriculture and provide the necessity of and justification for sterilization facilities for future eco-friendly horticultural facility complexes, the basis for calculation, and basic data. The limitations of this study are the failure to evaluate the correlation between fungi and bacteria, and the fact that the detection characteristics of fungi and bacteria according to the concentration of the nutrient solution were not identified. To compensate for these limitations, further research needs to be conducted. In addition, it is necessary to determine how factors such as detailed varieties, cultivation methods, emission time, and greenhouse specifications affect species detection or concentrations. This study on drainage recycling will facilitate sustainable horticulture by providing insights into the recycling of resources and a reduction in water pollutants, will highlight the improvement of horticulture facilities for sustainable agriculture, and provide the necessary justification for sterilization facilities for future eco-friendly horticultural facility complexes, the basis for calculation, and basic data.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12091340/s1, Video S1: Output video of drainage water in horticulture nutrient solution hydroponic system.

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Appendix A

Table A1. Species classification of fungi and bacteria based on the detection results obtained from 36 study sites.

Sites	Fungi							Bacteria						
	Ph	**Cl**	**Or**	**Fa**	**Ge**	**Sp**	**Td**	**Ph**	**Cl**	**Or**	**Fa**	**Ge**	**Sp**	**TD**
P1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P2	0	0	0	0	0	0	0	1	1	2	2	2	2	5
P3	1	1	2	2	2	3	10	1	1	1	1	1	1	6
P4	1	1	1	1	1	2	10	1	1	1	1	1	1	5
P5	3	3	4	5	5	8	28	1	1	2	2	2	2	5
P6	2	2	2	2	2	4	11	1	1	1	1	1	1	6
P7	2	2	2	2	2	5	8	1	1	1	1	1	1	2
P8	2	2	2	3	3	6	22	1	1	1	1	1	1	1
P9	0	0	0	0	0	0	0	1	1	1	1	1	2	6
P10	0	0	0	0	0	0	0	1	1	1	1	1	1	2
P11	1	3	4	4	4	4	12	1	2	2	2	2	3	8
P12	1	1	1	2	2	2	4	0	0	0	0	0	0	0
T1	0	0	0	0	0	0	0	1	1	1	1	1	1	3
T2	2	2	2	2	2	4	18	1	1	1	1	1	1	5
T3	2	2	2	3	3	5	19	1	1	1	1	1	1	1
T4	2	2	2	2	2	3	7	1	1	1	1	1	1	3
T5	1	1	1	1	1	2	11	1	1	1	1	1	1	5
T6	2	2	2	2	2	2	10	0	0	0	0	0	0	0
T7	0	0	0	0	0	0	0	1	1	1	1	1	1	1
T8	2	2	2	2	2	6	11	1	1	1	1	1	1	3
T9	3	3	3	3	3	6	13	1	1	1	1	1	1	1
T10	0	0	0	0	0	0	0	1	1	1	1	1	1	3
T11	0	0	0	0	0	0	0	1	1	1	1	1	1	3
T12	1	1	1	1	1	2	2	0	0	0	0	0	0	0
S1	2	3	3	3	3	4	17	1	2	2	2	2	2	5
S2	2	2	2	2	2	3	16	1	1	1	1	1	2	4
S3	2	2	3	3	3	4	15	1	2	2	2	2	4	8
S4	2	2	2	2	2	4	17	1	2	2	2	2	4	8
S5	1	1	1	1	1	3	11	1	2	2	2	2	3	8
S6	2	2	2	3	3	6	23	1	1	1	1	1	1	4
S7	2	3	3	3	3	5	19	1	1	1	1	1	1	4
S8	2	2	2	2	2	3	14	1	2	2	2	2	5	10
S9	2	3	4	5	5	9	30	1	2	2	2	2	4	15
S10	1	2	2	2	2	5	19	1	1	1	1	1	2	5
S11	3	4	4	4	4	6	15	1	1	1	1	1	1	1
S12	2	3	3	4	4	4	7	1	2	2	2	2	3	5

P: paprika, T: tomato, S: strawberry, Ph: phylum, Cl: class, Or: order, Ge: genus, Fa: family, Sp.: species, TD: total density.

Appendix B

Table A2. Detection of fungi and bacteria species lists from 36 study sites.

Species	Paprika												Tomato												Strawberry											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Fungi																																				
Ascomycota (Phylum)																																				
Dothideomycetes (Class)																																				
Pleosporales (Order)																																				
Pleosporaceae (Family)																																				
Alternaria (Generic)																																				
Alternaria sp. (*Species*)											1										3				1											1
Botryosphaeriales																																				
Botryosphaeriaceae																																				
Botryosphaeria																																				
Botryosphaeria sp.											3																				2				2	
Sordariomycetes																																				
Glomerellales																																				
Glomerellaceae																																				
Colletotrichum																																				
Colletotrichum sp.																											6						1			
Sordariomycetes																																				
Nectriaceae																																				
Fusarium																																				
Fusarium lactis							1	4																												
Fusarium oxysporum			3	4		3									4		5			1					4	5	2	6	4	2	3	4	1	4	1	
F. oxysporum f. sp. *cucum.*							1																													
Fusarium solani					2															1								1	1				2	2		
Fusarium sp.			4	6	2	4	4	4			4				4		6	5		3					6	6	6	6	6	5	5	6	5	6	6	2
Incertae sedis																																				

Table A2. *Cont.*

Species	Paprika												Tomato												Strawberry											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Incertae sedis																																				
Plectosphaerella																																				
Plectosphaerella cucumerina			3		5									3																						
Eurotiomycetes																																				
Eurotiales																																				
Trichocomaceae																																				
Penicillium																																				
Penicillium sp.																																	2			
Oomycota																																				
Peronosporales																																				
Peronosporaceae																																				
Phytophthora																																				
Phytophthora cactorum																														6			5			
Phytophthora capsici					6																															
Phytophthora cinnamomi								2																												
Phytophthora fragariae												1																						6		
Phytophthora nicotianae																																		1		2
Phytophthora sp.					6			2			4				1															6			6			
Basidiomycota																																				
Agaricomycetes																																				
Cantharellales																																				
Ceratobasidiaceae																																				
Rhizoctonia																																				
Rhizoctonia fragariae																															6				2	
Rhizoctonia solani					2																3										3				2	
Heterokontophyta																																				
Oomycota																																				

Table A2. *Cont.*

Species	Paprika												Tomato												Strawberry											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Peronosporales																																				
Pythiaceae																																				
Pythium																																				
Pythium aphanidermatum														6							1			1			1			1						
Pythium dissotocum					1	2	1	5							5	1				2	1															
Pythium irregulare														3																			2			
Pythium sp.					4	2	1	5				3		6	5	1				2	4			1	6	5		4		3		4	6		2	2
Pythium tracheiphyllum																				2	1															
Incertae sedis																																				
Incertae sedis																																				
Incertae sedis																																				
Olpidiaceae																																				
Olpidium																																				
Olpidium virulentus																5		5																		
Bacteria																																				
Proteobacteria																																				
Alphaproteobacteria																																				
Rhizobiales																																				
Rhizobiaceae																																				
Agrobacterium																																				
Agrobacterium tumefaciens											3		3	5											1		1	1	2			1	5			1
A. tumefaciens Ti-plasmid											3																		2			1				
Gammaproteobacteria																																				
Enterobacterales																																				
Pectobacteriaceae																																				
Pectobacterium																																				

Table A2. *Cont.*

Species	**Paprika**												**Tomato**												**Strawberry**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Erwinia carotovora subsp. *carotovora*		3			4	6														3	1															
Proteobacteria																																				
Gammaproteobacteria																																				
Pseudomonadales																																				
Pseudomonadaceae																																				
Pseudomonas																																				
Pseudomonas fluorescens		2	6	5	1		2	1	5	2	2				1	3	5		1			3	3			1	4	4	4	4	4	5	5	2	1	1
Pseudomonas marginalis									1																		1	1				1	1			
Pseudomonas viridiflava																									4	3	2	2				2	4	3		3

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