Influence of substrate temperature on microbial biodiversity in soilless cultures of 'Melen' cucumber

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Abstract: The aim of the study was to evaluate the influence of heating growth substrates (peat and coconut fibre) at night in different plant cultivation systems (slabs and cylinders) on the number of bacteria and filamentous fungi inhabiting those substrates in soilless cultivation of the cucumber variety 'Melen' in a greenhouse. The study assessed the effects of temperature in unheated substrates (below 17°C) and substrates heated to 17-20°C and 25-30°C. Heating the substrates to 25-30°C increased the total number and diversity of the isolated bacteria, including fluorescent *Pseudomonas* bacteria, in the peat substrate, and resulted in a greater number of spore-forming bacteria in the coconut fibre substrate. Larger numbers of fluorescent *Pseudomonas* bacteria were isolated from cylinders heated to a temperature of 25-30°C. There was no observable influence of substrate heating on the populations of filamentous fungi, actinomycetes, or bacteria of the genus *Azotobacter*.

Key-Words: *Cucumis sativus*; substrate heating; bacterial population; fungal population

1. Introduction

The number and activity of microorganisms inhabiting a growth substrate or the rhizosphere of plants depends on, among other things, the species of the plants and their physiological state, and the cultivation and environmental conditions such as: temperature (Smith 2005), composition of the atmosphere (Liesack et al. 2000), pH (Wakelin et al. 2008; Ausec et al. 2009), availability of nutrients and soil moisture content (Smith 2005). A change in the temperature of the substrate modifies important and complex interactions in the rhizosphere among microorganisms and between microorganisms and plants. Heating or cooling affects, either directly or indirectly, the size and species composition of microbial populations inhabiting the root zone of plants and the growth substrate.

Direct effects consist primarily in inhibiting the development of the species or groups of microorganisms for which a given temperature range is unsuitable, and in stimulating those microorganisms for which the new temperature ranges are more optimal for growth and greater metabolic activity such as atmospheric nitrogen fixation (Darbyshire 1972).

Indirect effects include interaction between plant roots and microorganisms (promoted, for example, by root secretions), changes in pH (Smith and Paul 1986), antagonism between microorganisms (Lengkeek and Otta 1979; Shanahan et al. 1992), or change in the populations of bacteriophagous or mycophagous nematodes (Sas Paszt et al. 2014; Yeates et al. 1993).

A change in temperature in soilless cultivation of plants can improve the economic aspect of growing vegetables under covers. In addition to increased vegetative growth and crop yield (Sas Paszt et al. 2014; Gosselin and Trudel 1985), heating the growth substrates can affect the physiological condition of plants

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by stimulating the development of beneficial microorganisms that colonize them while living in the rhizosphere, and by inhibiting the activity of pathogens. The temperature is an important factor because it also influences the effectiveness of biological plant protection products used in greenhouse cultivation (Boyle and Cutler 2012; Kope et al. 2008; Shanahan et al. 1992; Hannusch and Boland 1996; Fiedler and Sosnkowska 2007). Knowledge of the effects of substrate temperature will allow selection of appropriate products to stimulate plant growth and biological plant protection products containing beneficial microorganisms.

Earlier research conducted in a greenhouse, under similar conditions, had shown a favourable effect of heating cultivation substrates to 25-30°C on the vegetative growth and yield of cucumber plants of the cultivar 'Melen' (unpublished data). For this reason, the research was continued in terms of the influence of heating peat and coconut fibre substrates on the populations of microorganisms inhabiting them.

The aim of the study was to assess the influence of heating growth substrates at night on the number of bacteria and filamentous fungi inhabiting peat and coconut fibre substrates during cultivation of the cucumber variety 'Melen' in two cultivation systems: mats and cylinders.

2. Methodology

2.1. Experimental combinations

In an experiment conducted in 2013, two types of growth substrates were used: a commercially available coconut fibre substrate (Ceres International sp. z o.o.) and a peat substrate consisting of a mixture of highmoor peat 70%, bark 30%, complex fertilizer 1 kg .m-1, calcium nitrate 0.2 kg/m^3, chalk, pH 5.5 (Ceres International sp. z o.o.). The substrates were heated from midnight to 6 o'clock in the morning when the temperature at night was below 17°C. The experiment involved testing the effects of the following temperatures:

- Control, unheated substrate (below 17°C)
- Growth substrate heated with air blown in at a temperature of 17-20°C
- Growth substrate heated with air blown in at a temperature of 25-30°C

Plants of the cucumber variety 'Melen' were grown in two different types of containers: cylinders with a capacity of approx. 5.5 litres (Ø 20 cm, h = 18cm), or in cultivation mats (120 × 20 × 10 cm) with a capacity of approx. 24 litres. Irrigation and dosage of minerals (a mixture of calcium nitrate, magnesium sulfate, potassium sulfate, potassium phosphate, microelements, and nitric acid) were adapted to the prevailing weather conditions (temperature and insolation in the greenhouse), and the stage of plant growth. The pH of the nutrient media was dependent on the type of substrate in which the plants were grown, and was 6.8 for peat, and 5.7 for coconut fibre.

The experiment consisted of the following combinations:

1. **control** – cylinders with peat substrate, unheated (below 17°C)
2. cylinders with peat substrate heated to 17-20°C
3. cylinders with peat substrate heated to 25-30°C
4. **control** – mats with peat substrate, unheated (below 17°C)
5. mats with peat substrate heated to 17-20°C
6. mats with peat substrate heated to 25-30°C
7. **control** – cylinders with coconut fibre, unheated (below 17°C)
8. cylinders with coconut fibre heated to 17-20°C
9. cylinders with coconut fibre heated to 25-30°C
10. **control** – mats with coconut fibre, unheated (below 17°C)
11. mats with coconut fibre heated to 17-20°C
12. mats with coconut fibre heated to 25-30°C

2.2. Estimation of the number of microorganisms

Samples of the substrates for microbiological analyses were collected at monthly intervals:

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- August – beginning of experiment – initial analysis of substrates
- September – day 30 of plant cultivation
- October – day 60 of plant cultivation
- November – day 90 of plant cultivation – end of experiment

The samples of substrates from all the cylinders and mats were collected together with the roots growing in them, at approx. 50-100 g (to a depth of approx. 30 cm), and were mixed thoroughly. Then the samples were used to prepare bulk samples (each containing 10 g of substrate from each cylinder or mat). The bulk samples were again mixed thoroughly and suspended in sterile distilled water at a ratio of 1 : 9. The suspensions were homogenized with a BagMixer homogenizer (Interscience) for 10 minutes at 360 swings per minute. The homogenized suspensions were then used to prepare serial decimal dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}), which were used to inoculate the following culture media depending on the parameter to be estimated: the total number of bacteria was estimated on 10% tryptic soy agar (TSA) (Ghyselinck et al. 2013); total number of filamentous fungi was estimated on Rose Bengal Chloramphenicol Agar (Ryckeboer et al. 2003); total number of actinomycetes was estimated on 1.5% aqueous agar (Lingappa and Lockwood 1960); total number of bacteria corresponding morphologically to bacteria of the genus *Azotobacter* was estimated on Burk's medium (Bergey's Manual of Systematic Bacteriology Vol. 2B 2nd ed.) containing: K_2HPO_4 0.64 g, KH_2PO_4 0.2 g, $MgSO_4 \times 7H_2O$ 0.2 g, NaCl 0.2 g, $CaSO_4 \times 2H_2O$ 0.05 g, Na_2MoO_4 0.001 g, $FeSO_4$ 0.003 g, Mannitol 20 g, Agar 12 g, distilled water 1000 g; total number of fluorescent *Pseudomonas* bacteria was estimated on S1 medium (Gould et al. 1984) containing: sucrose 10 g, glycerol 12.6 g, Casamino acids 5 g, $NaHCO_3$ 1 g, $MgSO_4 \times 7H_2O$ 1 g, K_2HPO_4 2.3 g, SLS 1.2 g, trimethoprim 20 mg, agar 18 g, distilled water 1000 g; total number of spore-forming bacteria was estimated by incubating suspensions at 80°C for 30 minutes and plating them on 10% tryptic soy agar.

Plates were incubated for 72 hours at 30°C (S1 and Burk's media), for 10-14 days at 28°C (10% TSA, aqueous agar), and for 5-7 days at 25°C (Rose Bengal Chloramphenicol Agar). When calculating the number of microorganisms, only the plates on which the number of colonies fell within the range 30-300 were taken into consideration. The results were converted to colony-forming units per 1 gram of dry weight of medium (cfu * g^{-1} DW). In order to express the number of microorganisms in terms of per 1 g of dry weight, samples of the media were dried at 105°C for 18 hours.

2.3. Estimation of the activity and diversity of microorganisms

Determination of the activity and diversity of aerobic microorganisms was performed for uncultivated substrates used for establishing the experiment and those sampled on day 30 and day 90 of the experiment.

To estimate the activity and diversity of microorganisms colonizing the test substrates, EcoPlates (Biolog Inc.) were used. Test samples were prepared according to a modified procedure described by Gomez et al. (2006). The samples (10 g) were suspended in sterile distilled water (90 g) and homogenized using the BagMixer homogenizer for 10 minutes at 360 rpm. The homogenized suspensions were used to prepare serial decimal dilutions, which were incubated for 24 hours at room temperature (approx. 18-20°C). Subsequently, the EcoPlates were inoculated with 10^{-4} dilutions of the suspensions at 100 µl per well. The inoculated plates were incubated for seven days at room temperature. The results (optical density of the suspensions in the wells) were recorded every 24 hours for a wavelength of 590 nm, using a semi-automatic Biolog system equipped with an ELx808 reader (Biotek) and Microlog3 software (version 5.2.01). The final estimates of the activity of microorganisms were based on the results obtained after 96 hours.

Microbial activity, based on the activity of dehydrogenase enzymes, was estimated on the basis of the Average Well Color Development (AWCD) (Gomez et al. 2006; Choi and Dobbs 1999). The value of this parameter was calculated using the following formula:

$AWCD = \Sigma OD_i/31$, where: OD_i is the optical density of each well.

Microbial diversity was estimated by means of the Shannon-Weaver index (H):

$H= -\Sigma p_i(\ln p_i)$, where: p_i is the level of microbial activity in individual wells (OD_i)

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divided by the sum of the activity in all the wells (Σ OD_i). When assessing the level of microbial activity and the 'H' factor, the threshold value $OD = OD_i$ - OD of the control well was established (Gomez et al. 2006).

2.4. Estimation of the activity and diversity of microaerophilic / anaerobic microorganisms

Determination of the activity and diversity of microaerophilic and anaerobic microorganisms was performed on day 90 of the experiment.

To estimate the diversity of anaerobic and microaerophilic microorganisms, samples were prepared as above, with slight modifications. The samples were homogenized using the BagMixer homogenizer, diluted tenfold in freshly autoclaved and cooled distilled water, protected against oxygen ingress, and incubated at room temperature for 24 hours. EcoPlates were then inoculated with 10^{-3} dilutions of the suspensions at 100 µl per well. The inoculated plates were placed in an aerostat from which oxygen had been removed with a mixture of pyrogallol and sodium hydroxide in the amounts of 1 g pyrogallol and 1 g NaOH per 100 cm^3 of vessel volume (Willis 1977). The whole procedure of inoculating and placing the plates in the aerostat did not last longer than 30 minutes (Christian and Lind 2006). The aerostats with the plates were incubated for 7 days at room temperature. The results (optical density of the suspensions in the wells) were recorded after seven days for a wavelength of 590 nm, using a semi-automatic Biolog system equipped with an ELx808 reader (Biotek) and Microlog3 software (version 5.2.01).

The activity and diversity of microorganisms were estimated on the basis of AWCD and H values, respectively.

2.5. Statistical analysis

All of the results (number of microorganisms, AWCD, H) were subjected to uni- or multivariate analysis of variance using Statistica version 10 (Statsoft Inc., 2012).

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3. Results and discussion

3.1. Effect of substrate on the number, activity and diversity of microorganisms

Analysis of uncultivated substrates used to establish the experiment showed that they differed in terms of the number, activity (AWCD) and diversity (H) of the microorganisms living in them. Compared with the coconut fibre substrate, the peat substrate was inhabited by a larger and more diverse population of bacteria and actinomycetes as well as filamentous fungi. On the other hand, the coconut substrate had a larger population of spore-forming bacteria and was characterized by higher microbial activity (Tables 1, 2). No fluorescent *Pseudomonas* bacteria were found in the coconut substrate. Neither of the substrates contained any bacteria corresponding morphologically to the genus *Azotobacter* (Table 1).

The difference in the size of microbial populations in the two types of substrate is most likely associated with their different pH values and different levels of nutrients and their availability to microorganisms. The chemical analyses indicate that the peat substrate contains approx. 76.2% organic matter at a pH of 4.9, whereas the coconut fibre substrate approx. 55.2% at pH 6.7 (Sas Paszt et al. 2014). Similar results concerning differences in the activity of dehydrogenase enzymes and the total number of bacteria, fungi and actinomycetes occurring in uncultivated peat and coconut fibre substrates were obtained by Kleiber et al. (2012) and Sas Paszt et al. (2014) in their work with tomato plants. Martinez et al. (2013) reported a greater number of bacteria of the genus *Bacillus* (spore-forming bacteria) in a coconut fibre substrate (pH 5.92) and a larger population of fluorescent *Pseudomonas* bacteria in a peat substrate (pH 5.83).

The substrate samples analyzed every 30 days revealed increased activity of microorganisms and larger populations of all the tested groups of bacteria, with the exception of actinomycetes inhabiting peat substrates (Tables 1 and 4). The largest populations of the studied bacterial groups were observed after 30 or 60 days from the beginning of cultivation, whereas the populations of fungi were largest after 30 days in the peat substrate and after 90 days in the coconut fibre substrate. Compared with peat, the coconut fibre substrate was characterized by larger populations of all the studied groups of microorganisms with the exception of actinomycetes (Table 1).

Data available in the literature does not provide clear information on the dynamics of increase in the number of individual groups of microorganisms or the total number of bacteria and fungi during cultivation of plants under greenhouse conditions (Kleiber et al. 2012, Koohakan et al. 2004, Sas Paszt et al. 2014, Khalil et al. 2000). On the one hand, Kleiber et al. (2012) reported a reduction in the number of bacteria and actinomycetes with a simultaneous increase in the populations of fungi in cultivated peat and coconut substrates in comparison with the populations of these microorganisms prior to cultivation. On the other hand, in a study with strawberry plants, Martinez et al. (2013) reported an increase in the number of bacteria, including fluorescent *Pseudomonas* bacteria, *Bacillus* (spore-producing bacteria) and actinomycetes, with a simultaneous decrease in the number of fungi in cultivated peat and coconut substrates compared with initial populations. The same authors observed almost twice the number of fluorescent *Pseudomonas* bacteria in a cultivated peat substrate, and a forty-seven times larger population of *Bacillus* bacteria in a cultivated coconut substrate in relation to uncultivated substrates. Sammar and Alsanius (2001), in turn, registered increased growth in the populations of fungi and bacteria, including actinomycetes and fluorescent *Pseudomonas* bacteria, in the rhizosphere of plants cultivated in a peat substrate, whereas Koohakan et al. (2004) noted a decrease in the number of fluorescent *Pseudomonas* bacteria isolated from tomato roots already after 5 weeks from the beginning of cultivation in a soilless system that also included a coconut substrate.

In the case of dehydrogenase enzymes, Kleiber et al. (2012) reported a decrease in the activity of these enzymes during the course of an experiment in a cultivated coconut substrate and an increase in their activity in a peat substrate.

In our study, the diversity of the bacteria inhabiting the peat substrate

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significantly declined during the course of the experiment (Table 4).

There was no observable effect of the type of substrate on the diversity of microaerophilic and anaerobic bacteria, or on the activity of dehydrogenase enzymes produced by them (Table 4).

3.2. Effect of substrate heating on the number, activity and diversity of microorganisms

Differences were found in the development of the populations of microorganisms inhabiting the cultivated peat and coconut fibre substrates and in their activity following the use of heating. In comparison with the unheated substrates, the largest differences in the abundance of the studied groups of bacteria were observed in the substrates heated to 25-30°C (Tables 2, 3, 5, 6). The observed changes in the heated substrates were probably caused by the creation of near-optimal conditions for the growth and development of the studied groups of bacteria and fungi. Pietika et al. (2004) have indicated that an optimum temperature for the growth and respiration of most bacteria and fungi colonizing cultivated soils or humus soils is a temperature in the range 25-30°C. This relationship is confirmed by the results of our study.

Heating the peat substrate to a temperature of 25-30°C resulted in an increase in the total population of the isolated bacteria, including fluorescent *Pseudomonas* bacteria. In the case of the other groups of microorganisms studied, there were no statistically significant long-lasting differences in their numbers (Table 3). Literature information on the influence of temperature on the number of fluorescent *Pseudomonas* bacteria is difficult to interpret. Apart from temperature, the population size of these bacteria largely depends on the type of substrate, the properties of specific strains and their interaction with micro- and macrofauna present in the substrate, and the species of the cultivated plant. O'Callaghan et al. (2001), in a study of changes in the number of *Pseudomonas fluorescens* (strain CHAO-Rif) in the soil, observed a lower survival rate of these bacteria in the rhizosphere of potato plants at a temperature of 20°C, compared with those in the rhizosphere of plants growing at lower temperatures (10-15°C). Loper et al. (1985) and Davies and Withbread (1989), while conducting experiments on the abundance of beneficial strains of *P. fluorescens* in the rhizosphere of potato and radish, had earlier noted that the bacteria differed in terms of survival in the soil and the rate of root colonization depending on the temperature of the growth substrate. Results similar to those of the above authors were also obtained by Schmidt et al. (2004), who observed a reduction in the number of *P. fluorescens* bacteria (strain B5) and differences in their distribution on the roots of sugar beet at a soil temperature in the range 25-35°C, compared with soils at a lower temperature.

Heating the peat substrate did not produce statistically significant effects on the other groups of microorganisms examined in our study (Table 3).

In terms of microbial activity, the heating of cylinders and mats containing the peat substrate increased the activity of aerobic microorganisms inhabiting them on day 30 (cylinders) and 90 (mats) of the experiment (Table 6). There was also a greater diversity of aerobic microorganisms in the heated cylinders with the peat substrate (Table 6). In the case of microaerophilic and anaerobic bacteria, the use of heating resulted in a decrease in their activity and biodiversity (Table 6). The influence of temperature on the diversity of microorganisms inhabiting the soil or growth substrates has also been estimated on the basis of phospholipid fatty acid analyses – PLFA (Ranneklev and Baath 2003, Pettersson and Baath 2003). Analyses of phospholipids extracted from the soil indicated a greater diversity of microorganisms in the soil at 30°C compared with the soil at 5 and 20°C (Pettersson and Baath 2003).

In the case of microbial populations inhabiting coconut fibre, heating the substrate to 25-30°C resulted in an increase in the number of the isolated spore-forming bacteria (Table 2). Literature data indicate that a temperature of approx. 30°C promotes their development as opposed to lower temperatures. Reddy and Rahe (1989) had observed that the strain of *Bacillus* investigated by them was present in greater numbers in the soil at a temperature of 22-25°C than at a temperature of 17-19°C. Ryu et al. (2005) observed a larger population of *Paenibacillus polymyxa* on the roots of cucumber plants

growing at a temperature of 30°C compared with plants grown at 20°C. Similar results were obtained by Melent'ev et al. (2000), who observed that with increasing temperature, the populations of *Bacillus* strains studied by them also increased.

Heating the coconut fibre substrate had no statistically significant effect on the other groups of microorganisms examined in our study (Table 2).

A brief increase was observed in the activity of aerobic microorganisms inhabiting the coconut fibre mats heated to 25-30°C on day 90 of the experiment. No similar effect was registered in the case of microorganisms inhabiting the cylinders. The coconut fibre substrate from the cylinders heated to 25-30°C was characterized by a greater index of microbial diversity on day 30 of the experiment compared with the control substrate. An increase was noted in the activity of anaerobic and microaerophilic microorganisms inhabiting the coconut fibre substrate; there were no significant differences in their diversity (Table 5).

3.3. Effect of cultivation system on the number, activity and diversity of microorganisms

There was a larger number of fluorescent *Pseudomonas* bacteria in all three temperature ranges tested and of spore-forming bacteria at a temperature of 25-30°C isolated from cylinders with the coconut fibre substrate (Table 2).

In the case of the peat substrate, there was a greater number of spore-forming bacteria at a temperature of 17-20°C and 25-30°C, and of filamentous fungi at a temperature of 17-20°C isolated from the mats, and a greater number of fluorescent *Pseudomonas* bacteria at a temperature of 25-30°C isolated from the cylinders (Table 3).

Different results had been obtained by Sas Paszt et al. (2014), who reported no observable influence of the cultivation system on the number of microorganisms in the rhizosphere of tomato plants of the variety 'Tamaris'. The differences in the size of the populations of some groups of microorganisms may have resulted from different conditions within the growth containers, such as lower availability of oxygen to the roots of plants and microorganisms in the mats than in the cylinders.

4. Conclusions

The type of growth substrate had a significant influence on the abundance of the various groups of microorganisms inhabiting them. Heating the substrates to 25-30°C had the greatest effect on changing the population size, activity, and biodiversity of the studied groups of bacteria. Heating the peat substrate to 25-30°C increased the total population of the isolated bacteria, including fluorescent *Pseudomonas* bacteria. Heating the coconut fibre substrate to 25-30°C resulted in a significant increase in the number of the isolated spore-forming bacteria. There was no significant influence of temperature on the abundance of filamentous fungi in the test substrates.

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Tables

Table 1. Effect of peat and coconut fibre substrates on the number of microorganisms inhabiting them in soilless cultivation of cucumber variety 'Melen' (average for all temperatures)

Parameter	Sampling times during cultivation	Number of microorganisms $\times 10^6$ cfu·g^{-1} DW	
		Peat	Coconut fibre
Total number of bacteria	Beginning	293 b	180 a
	Day 30	1673 a	5151 b
	Day 60	3350 a	4819 b
	Day 90	3048 a	2426 a
Total number of spore-forming bacteria	Beginning	0.04 a	0.25 b
	Day 30	0.12 a	1.69 b
	Day 60	0.3 a	1.63 b
	Day 90	0.3 a	1.11 b
Total number of fluorescent *Pseudomonas* spp.	Beginning	0.05 b	0* a
	Day 30	3.98 a	150.24 b
	Day 60	1.41 a	2.26 a
	Day 90	4.94 a	9.93 b
Total number of actinomycetes	Beginning	32.67 b	1.3 a
	Day 30	54.76 b	6.59 a
	Day 60	0.45 a	1.42 b
	Day 90	2.67 a	9.71 a
Total number of bacteria morphologically resembling *Azotobacter* spp.	Beginning	0*	0*
	Day 30	0.63 a	164.85 b
	Day 60	6.7 a	115.7 b
	Day 90	10.97 a	29.02 a
Total number of fungi	Beginning	14.1 b	6 a
	Day 30	20.29 a	29.1 b
	Day 60	18.2 a	26.68 b
	Day 90	19.03 a	30.36 b

Results of microbiological analyses verified by two-way analysis of variance (Statistica 10).
Homogeneous groups determined by Tukey's HSD test at $\alpha = 0.05$. * – below detection limit

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Table 2. Effect of temperature and cultivation system on the number of microorganisms inhabiting coconut fibre substrates during cultivation of cucumber variety 'Melen'

Parameter	Sampling times during cultivation	Number of microorganisms × 10^6 cfu·g^{-1} DW					
		Coconut fibre – mats			Coconut fibre – cylinders		
		Control	17-20°C	25-30°C	Control	17-20°C	25-30°C
Total number of bacteria	Beginning	180					
	Day 30	5390 b	4717 ab	7918 c	4028 a	4335 ab	4519 ab
	Day 60	2938 a	3351 ab	2288 a	7791 c	6987 bc	5563 a-c
	Day 90	2753 bc	2423 a-c	2057 ab	3043 c	2358 a-c	1925 a
Total number of spore-forming bacteria	Beginning	0.25					
	Day 30	0.93 a	1.60 b	1.14 ab	1.28 ab	1.00 a	4.18 c
	Day 60	1.62 a	1.42 a	1.62 a	1.62 a	1.72 a	1.76 a
	Day 90	1.57 b	0.85 a	0.84 a	0.81 a	1.16 ab	1.42 b
Total number of fluorescent *Pseudomonas* spp.	Beginning	0*					
	Day 30	145.73 bc	77.33 a	120.83 b	274.37 d	126.57 b	156.61 c
	Day 60	0.9 a	1.73 ab	1.8 ab	2.2 b	6.07 c	0.86 a
	Day 90	1.04 a	4.54 ab	16.2 d	12.14 cd	10.14 bc	15.5 cd
Total number of actinomycetes	Beginning	1.30					
	Day 30	0* a	23.71 c	2.31 ab	0* a	0.52 a	13.05 bc
	Day 60	0* a	0* a	2.57 b	1.58 ab	2.43 b	1.96 b
	Day 90	2.78 a	0* a	1.8 a	40.45 b	3.47 a	9.79 a
Total number of bacteria morphologically resembling *Azotobacter* spp.	Beginning	0*					
	Day 30	254.44 d	149.5 a-c	215.94 cd	93.22 a	171.65 bc	104.4 ab
	Day 60	83.27 a	110.83 a	172.24 b	98.49 a	126.57 ab	102.77 a
	Day 90	0* a	0* a	0* a	52.76 ab	121.37 b	0* a
Total number of fungi	Beginning	6.00					
	Day 30	22.44 a	25.78 ab	34.45 b	29.55 ab	34.68 b	27.73 ab
	Day 60	19.43 a	21.39 a	23.14 ab	31.66 bc	36.76 c	27.73 a-c
	Day 90	35.85 a	27.84 a	36.5 a	30.25 a	27.74 a	23.98 a

Notes: As for Table 1.

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Table 3. Effect of temperature and cultivation system on the number of microorganisms inhabiting peat substrates during cultivation of cucumber variety 'Melen'

Parameter	Sampling times during cultivation	Number of microorganisms × 10^6 cfu·g^{-1} DW					
		Peat – mats			Peat – cylinders		
		Control	17-20°C	25-30°C	Control	17-20°C	25-30°C
Total number of bacteria	Beginning	293					
	Day 30	1306 ab	1844 bc	1611 ab	1723 b	926 a	2629 c
	Day 60	2775 a	2854 a	4856 b	2775 a	1902 a	4940 b
	Day 90	2220 ab	2766 bc	6019 d	2192 ab	1877 a	3214 c
Total number of spore-forming bacteria	Beginning	0.04					
	Day 30	0.17 c	0.15 bc	0.17 c	0.1 ab	0.07 a	0.08 a
	Day 60	0.17 a	0.23 a	0.2 a	0.11 a	0.88 b	0.22 a
	Day 90	0.3 ab	0.43 bc	0.62 c	0.13 a	0.11 a	0.18 ab
Total number of fluorescent *Pseudomonas* spp.	Beginning	0.05					
	Day 30	2.11 ab	1.34 ab	3.58 b	0.12 a	8.88 c	7.84 c
	Day 60	1.01 b	0.24 a	1.97 c	0.05 a	0.11 a	5.05 d
	Day 90	0.44 a	0.73 a	5.82 b	0.3 a	0.3 a	12.88 c
Total number of actinomycetes	Beginning	32.67					
	Day 30	91.41 c	87.82 c	51.46 b	74.75 c	16.48 a	6.64 a
	Day 60	0* a	1.32 c	0.22 ab	0* a	1.01 bc	0.13 ab
	Day 90	6.53 a	0* a	6.71 a	1.27 a	0.13 a	1.33 a
Total number of bacteria morphologically resembling *Azotobacter* spp.	Beginning	0*					
	Day 30	0* a	0* a	0* a	3.8 a	0* a	0* a
	Day 60	4.35 a	1.54 a	29.09 b	2.41 a	0.76 a	2.26 a
	Day 90	0* a	65.86 b	0* a	0* a	0* a	0* a
Total number of fungi	Beginning	14.1					
	Day 30	21.98 bc	20.64 bc	22.15 bc	25.72 c	14.71 a	16.6 ab
	Day 60	21.98 ab	26.35 b	23.94 b	11.02 a	10.9 a	15.01 ab
	Day 90	26.77 b	20.42 ab	19.24 ab	17.23 a	16.86 a	13.68 a

Notes: As for Table 1.

Table 4. Effect of substrate on the activity (AWCD) and diversity (Shannon-Weaver index) of microorganisms inhabiting peat and coconut fibre substrates during cultivation of cucumber variety 'Melen' (average for all temperatures).

Parameter	Sampling times during cultivation	Activity / Diversity of microorganisms	
		Peat	Coconut fibre
Aerobic bacteria AWCD	Beginning	0.15 a	0.53 b
	Day 30	1.39 a	1.47 a
	Day 90	0.71 a	0.9 b
Aerobic bacteria Shannon-Weaver index (H)	Beginning	5.95 b	1.91 a
	Day 30	2.37 a	2.32 a
	Day 90	2.78 a	3 b
Anaerobic / Microaerophilic bacteria AWCD	Day 90	0.65 a	0.63 a
Anaerobic / Microaerophilic bacteria Shannon-Weaver index (H)	Day 90	2.66 a	2.91 a

Results of microbiological analyses verified by one-way analysis of variance (Statistica 10).
Homogeneous groups determined by Tukey's HSD test at $\alpha = 0.05$.

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Table 5. Effect of temperature and cultivation system on the activity (AWCD) and diversity (Shannon-Weaver index) of microorganisms inhabiting coconut fibre substrates during cultivation of cucumber variety 'Melen'

Parameter	Sampling times during cultivation	Activity / Diversity of microorganisms					
		Coconut fibre – mats			Coconut fibre – cylinders		
		Control	17-20°C	25-30°C	Control	17-20°C	25-30°C
Aerobic bacteria AWCD	Beginning	0.53					
	Day 30	1.45 a	1.41 a	1.42 a	1.61 a	1.39 a	1.53 a
	Day 90	0.9 a	0.81 a	1.11 b	0.91 a	0.81 a	0.85 a
Aerobic bacteria Shannon-Weaver index (H)	Beginning	1.91					
	Day 30	2.6 b	1.75 a	1.96 a	1.8 a	3.07 c	2.76 bc
	Day 90	2.95 ab	2.98 ab	2.92 a	3.05 bc	3.01 a-c	3.1 c
Anaerobic / Microaerophilic bacteria AWCD	Day 90	0.48 a	0.49 a	0.81 c	0.57 ab	0.73 bc	0.68 bc
Anaerobic / Microaerophilic bacteria Shannon-Weaver index (H)	Day 90	2.89 ab	3.15 b	2.58 a	2.87 ab	2.89 ab	3.1 b

Results of microbiological analyses verified by two-way analysis of variance (Statistica 10).
Homogeneous groups determined by Tukey's HSD test at $\alpha = 0.05$.

Table 6. Effect of temperature and cultivation system on the activity (AWCD) and diversity (Shannon-Weaver index) of microorganisms inhabiting peat substrates during cultivation of cucumber variety 'Melen'

Parameter	Sampling times during cultivation	Activity / Diversity of microorganisms					
		Peat – mats			Peat – cylinders		
		Control	17-20°C	25-30°C	Control	17-20°C	25-30°C
Aerobic bacteria AWCD	Beginning	0.15					
	Day 30	1.66 b	1.34 ab	1.32 ab	0.99 a	1.45 ab	1.56 b
	Day 90	0.48 a	0.72 b	1.05 c	0.87 bc	0.44 a	0.71 b
Aerobic bacteria Shannon-Weaver index (H)	Beginning	5.95					
	Day 30	3.18 b	2.87 b	2.97 b	1.38 a	1.03 a	2.8 b
	Day 90	2.8 ab	2.9 b	2.93 b	2.54 a	2.53 a	2.97 b
Anaerobic / Microaerophilic bacteria AWCD	Day 90	0.75 b	0.64 b	0.43 a	0.93 c	0.43 a	0.68 b
Anaerobic / Microaerophilic bacteria Shannon-Weaver index (H)	Day 90	2.95 c	2.93 c	1.66 a	3.05 c	2.49 b	2.88 c

Notes: As for Table 5.

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