

The Influence of Heated Soil in Crop of “Tamaris” Tomato Plants on the Biological Activity of the Rhizosphere Soil

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Received 3 February 2014; revised 2 March 2014; accepted 9 March 2014

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Abstract

The aim of this study was to evaluate the effect of heated soil on the population of bacteria, fungi and nematodes inhabiting the soil of tomato cultivar “Tamaris” growing in peat and coconut substrates. In addition, these media were tested in two different containers: cylinders and slabs. The total number of bacteria and fungi was significantly higher in the peat substrate than in the coconut substrate. A much higher number of beneficial bacteria producing spores were noted in the coconut soil. The total number of bacteria and fungi was modified by the heating levels. In all the experimental treatments, most of the identified individuals belonged to the group of nematodes that feed on bacteria and it was the most diverse group of species. The highest total number of nematodes was recorded for the second heating level, with the exception of cultivation carried out in peat slabs, where the most of nematodes were found at the control level. The highest first crop was produced by plants growing on the peat slabs supplied with the highest soil temperature. Tomato roots of all the control treatments showed the highest root morphological parameters.

Keywords

Heated Soil; Root Morphology; Tomato; Peat; Coconut Fibre; Cylinder; Slab; Bacteria; Fungi; Nematodes

1. Introduction

Tomato is a plant with high heat requirements and sensitive to cold weather and frost. The optimum temperature for the growth of tomato plants is between 21°C and 27°C during the day and between 17°C and 21°C at night

How to cite this paper: Paszt, L.S., Trzciński, P., Bakalarska, M., Hołownicki, R., Konopacki, P. and Treder, W. (2014) The Influence of Heated Soil in Crop of “Tamaris” Tomato Plants on the Biological Activity of the Rhizosphere Soil. *Advances in Microbiology*, 4, 191-201. <http://dx.doi.org/10.4236/aim.2014.44025>

[1]. The soil temperature is very important for plant growth. The optimum soil temperature for tomato cultivation should be within the range 15°C - 18°C. The proper development of the root system depends on the optimal temperature of the soil. A temperature below 14°C reduces and inhibits the growth of the root system and encourages the development of fungal and bacterial diseases. However, a soil temperature higher than the air temperature promotes excessive vegetative growth, causing delays in flowering, and even shedding of buds and fruits, especially these from the first cluster.

The rhizosphere is a region of intense microbial activity and all nutrients that plants absorb from soil must pass through this region. Changes in the rhizosphere might affect the rhizosphere microflora and, in turn, plant growth. Soil microorganisms are the first to respond to environmental changes. Therefore, for an overall assessment of changes taking place in a heated soil, the total numbers of bacteria, fungi and actinomycetes were subjected to detailed studies at different periods of plant development [2]. The need for this research was due to the fact that any change in a physicochemical parameter of soil is reflected as a change in the processes that stimulate the biological activity of the soil. The studies done so far in a heated substrate show that the biological activity of soil microorganisms changed significantly as a result of the thermal factor. An evaluation of soil activity was carried out for “Tamaris” tomato plants at different heating temperatures supplied to the soil [3]. The biological activity of the rhizosphere soil was determined at four stages of plant development: at the beginning of flowering (V-May), at the beginning of fruiting (VI-June), during full fruiting (VII-July) and at the end of experiment (X-October).

The experiment was established in a greenhouse of the Research Institute of Horticulture in Skierniewice in April 2012 and completed in October 2012. The aim of this study was to evaluate the influence of heated soil (at three heating temperatures) on the population of bacteria, fungi and nematodes inhabiting the rhizosphere soil of tomato plants of the cultivar “Tamaris” growing in peat and coconut substrates [4] [5].

2. Materials and Methods

2.1. Experimental Treatment

The peat and coconut substrates were tested at the following heating levels:

1st level without heating—**control level**, unheated substrate.

2nd heating level—air temperature applied to a substrate was between 17°C and 20°C.

3rd heating level—air temperature applied to a substrate was set in the range from 25°C to 30°C.

The heating was turned off when the night temperature exceeded 17°C.

The experiment was carried out in 12 treatments and in 3 replications (one slab was one replication). Tomato plants cv. *Tamaris* were growing in peat and coconut substrates. These soils were tested in two different types of containers (see **Figure 1**): cylinders (each of 5 L capacity) and slabs (each of 20 L capacity).

Plant nutrition was done automatically. The amount and frequency of dosing the medium varied in different periods and phases of the growth of tomato plants and depended on the external conditions such as sunlight and air temperature. The mixtures of fertilizers such as calcium nitrate, potassium, magnesium sulfate, potassium sulfate, potassium phosphate, microelements and nitric acid were used for feeding plants.

The plant nourishment was controlled by electrical conductivity—EC and pH. The experiment included the following experimental treatments:

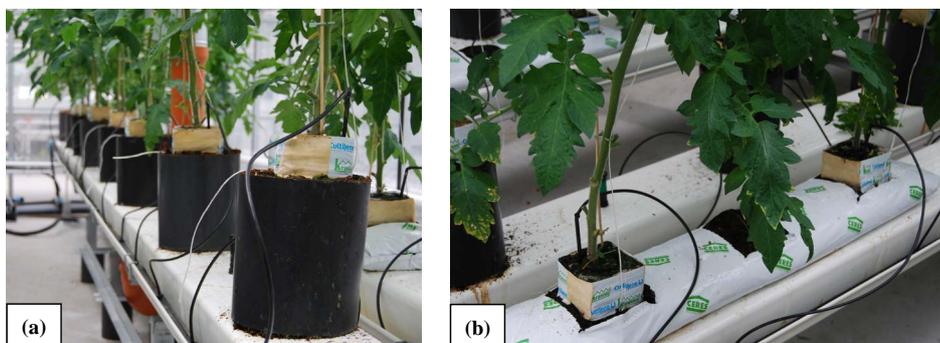


Figure 1. Tomato plants growing in (a) cylinders and in (b) slabs.

- 1) L1-1st heating level—**control treatment**—cylinders with peat substrate.
- 2) L2-2nd heating level—cylinders with peat substrate.
- 3) L3-3rd heating level—cylinders with peat substrate.
- 4) L1-1st heating level—**control treatment**—slabs with peat substrate.
- 5) L2-2nd heating level—slabs with peat substrate.
- 6) L3-3rd heating level—slabs with peat substrate.
- 7) L1-1st heating level—**control treatment**—cylinders with coconut substrate.
- 8) L2-2nd heating level—cylinders with coconut substrate.
- 9) L3-3rd heating level—cylinders with coconut substrate.
- 10) L1-1st heating level—**control treatment**—slabs with coconut substrate.
- 11) L2-2nd heating level—slabs with coconut substrate.
- 12) L3-3rd heating level—slabs with coconut substrate.

2.2. Yielding of Plants

In the vegetation period, plant yield was recorded and calculated per number of plants [6].

2.3. Determination of Root Growth and Morphological Parameters

- fresh and dry root weight [7].
- root morphological parameters (total root length, root diameter, root volume, root surface area and total number of root tips)—image analysis system with an Epson scanner, controlled by WinRhizo software (Regent Instruments Inc.).

2.4. Microbiological Analysis of Soil

Dynamics of development of selected microbial groups was determined at four monthly intervals, depending on the development phase of tomato:

- V-May, beginning of flowering.
- VI-June, beginning of fruiting.
- VII-July, full fruiting.
- X-October, last harvest and removal of plants.

In bulk samples collected from the rhizosphere of all experimental treatments, the numbers of microorganisms were determined using the plate method on selective agar media (in four replications). Mean number of colonies were given per fresh matter of a given substrate:

- total bacterial number with the number of bacteria producing spores were determined on a nutrient agar medium after 5 - 10 days of incubation at 28°C.
- total fungal count was determined on Rose Bengal Chloramphenicol Agar medium after 5 - 7 days of incubation at 25°C.
- the number of actinomycetes were determined on actinomycetes Isolation Agar and Water Agar media after 14 - 21 days of incubation at 26°C.

To calculate the number of microorganisms only the plates with colonies between 30 and 300 CFU were taken into account.

2.5. Nematological Analysis of Soil

Samples consisted of 100 g of soil with roots. Because of the significant share of roots in a sample, the material had to be cut into smaller pieces. Nematodes were isolated by the Baermann method on a sieve for 48 hours. The isolated nematodes were preserved in 20 ml of TAF mixture.

2.6. Statistical Analysis

The results were statistically evaluated (analyzed by) with multivariate analysis of variance verifying the hypothesis of equality of means ($H_0: x_1 = x_2 = x_3$) at significance level 0.05. It was done by Statistica software, version 10 (StatSoft Inc., 2012).

3. Results and Discussion

3.1. Crop

The very first harvest of tomatoes was about 30 days after flowering. Plants from the third heating level were the first that cropped. The tomato plants growing on the peat substrate produced the first crop (e.g. the number of fruits produced during the first week of cropping) earlier than the plants planted in the coconut substrate. The highest first crop was produced by plants growing on the peat slabs supplied with the highest soil temperature (the 3rd heating level, see **Figure 2**). This indicates a positive influence of peat as a growth substrate inserted in slabs on the yielding potential of tomato plants. The effect was due to the significantly higher levels of organic matter, N-NO₃ (168 mg/dm³), N-NH₄ (123 mg/dm³), and high levels of P (164 mg/dm³), Ca (727 mg/dm³) and microelements (Fe-2.6 mg/dm³, Mn-5,1 mg/dm³) in comparison to coconut.

The total yield (the yield from the whole experimental period) didn't differ significantly for all experimental treatments (see **Figures 3(a)** and **(b)**). However, it could be noticed that the crop of tomato plants growing in the cylinders filled with coconut fibre and slabs filled with peat slightly increased with increasing soil temperature. This indicates that irrespective of the growth substrate, the higher temperature increased the nutritional status of the plants, their shoot growth parameters, green colour of the leaves and subsequent yielding. Tomato plants growing in cylinders with peat and in slabs with coconut fibre gave comparable total yield for all heating levels. The tomato plants planted in coconut cylinders and in peat slabs at the third heating level gave the highest total yields. It is therefore suggested that the highest temperature of 25°C (higher by about 15°C at the 3rd heating level in comparison with the control: 10°C) had the most accelerating influence on the yielding potential of the plants. This was due to the enhanced nutrient uptake and acquisition, and higher energy and water supply to the plants.

3.2. Root Morphological Analysis

The soil temperature didn't have a significant impact on the growth of the tomato root system (**Figure 4(a)**). Although, tomato roots of all the control treatments showed the highest root morphological parameters. **Figure 4** presents only the results of the root volume, but the same results were also obtained for the root surface area, the root diameter and the root fresh weight. The statistical analysis showed that there was the significant influence of the container shape and the interaction between the container shape and the substrate type on the root system growth (**Figures 4(b)** and **(d)**). Tomato roots growing in peat cylinders were significantly longer, had a bigger volume and surface area than those of the roots growing in peat and coconut slabs (**Figure 4(d)**). This indicates that in comparison with slabs, the cylinder-shaped containers provided better aeration conditions to the plants and allowed the plants to grow better and form roots with a greater length, volume and surface area. It was also clearly visible that in the cylinders tomato roots grew horizontally (according to the natural geotropism), whereas in the slabs root growth was directed more vertically with a lower oxygen supply. Therefore, the root

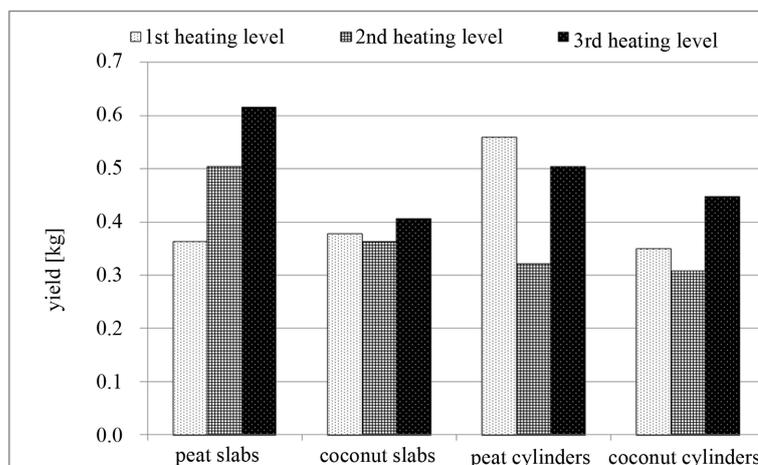


Figure 2. The first weekly crop of tomato plants growing in a greenhouse against the heating level.

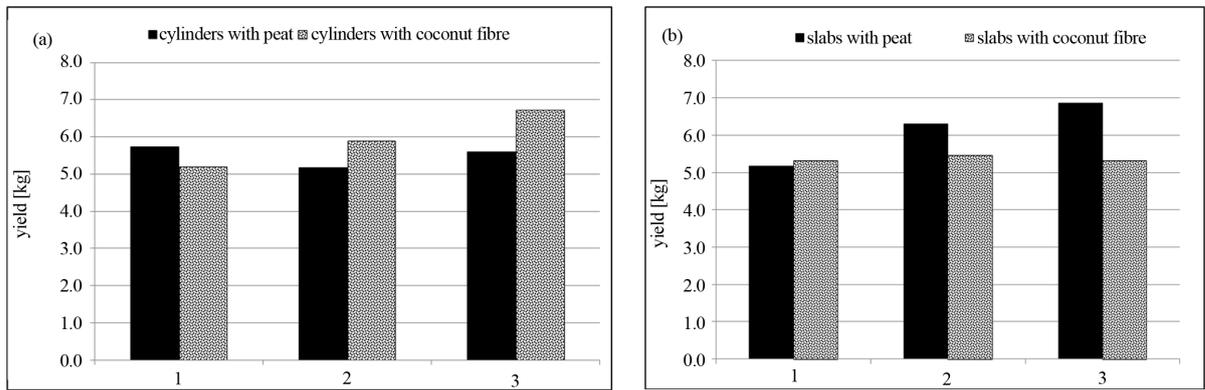


Figure 3. Total yield produced by tomato plants growing in (a) cylinders and (b) slabs against the heating level. 1—Control level, 2—second heating level, 3—third heating level.

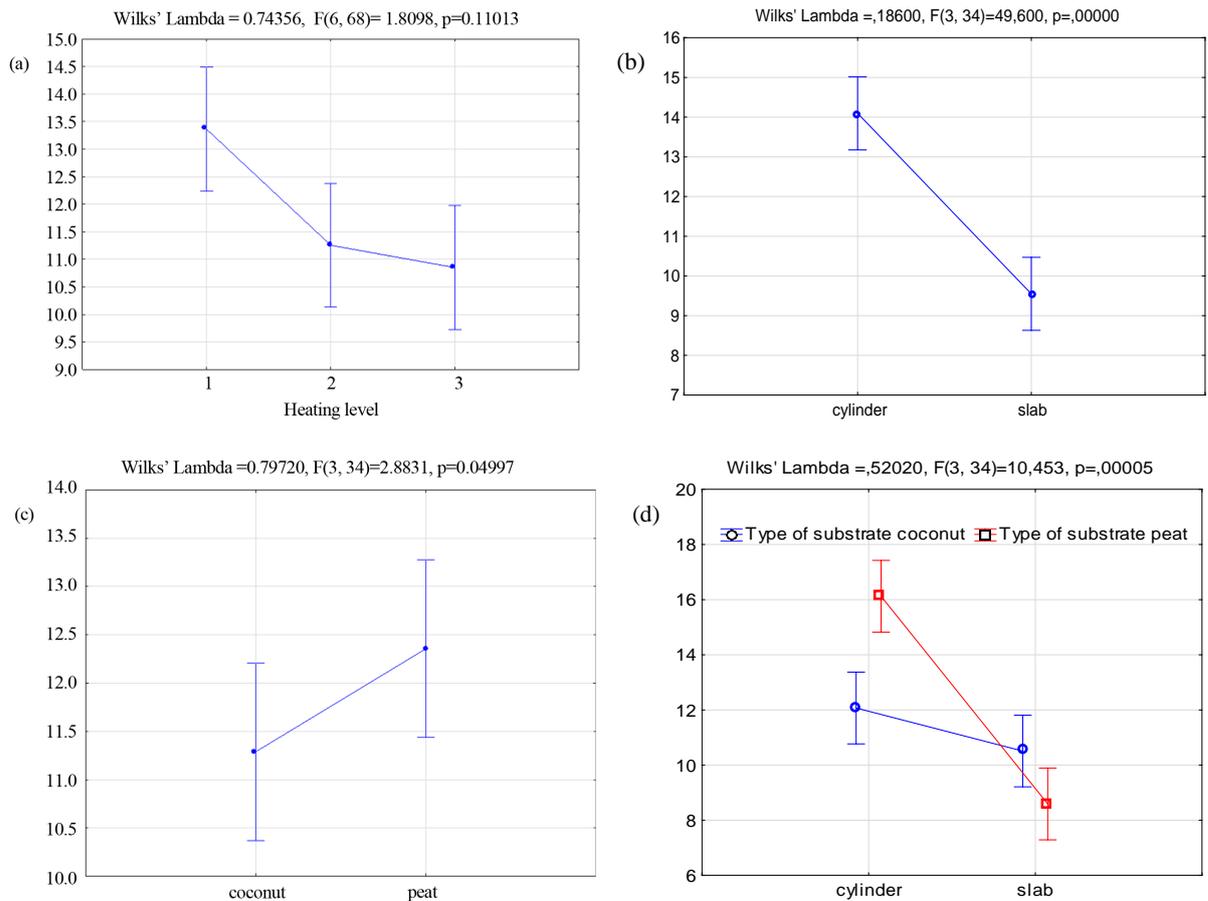


Figure 4. Expected marginal means of root volume [cm^3/L of soil] for the experimental factors: heating level (a), container shape (b), substrate type (c), container shape*substrate type (d); decomposition of effective hypotheses: columns mean 0.95 confidence intervals.

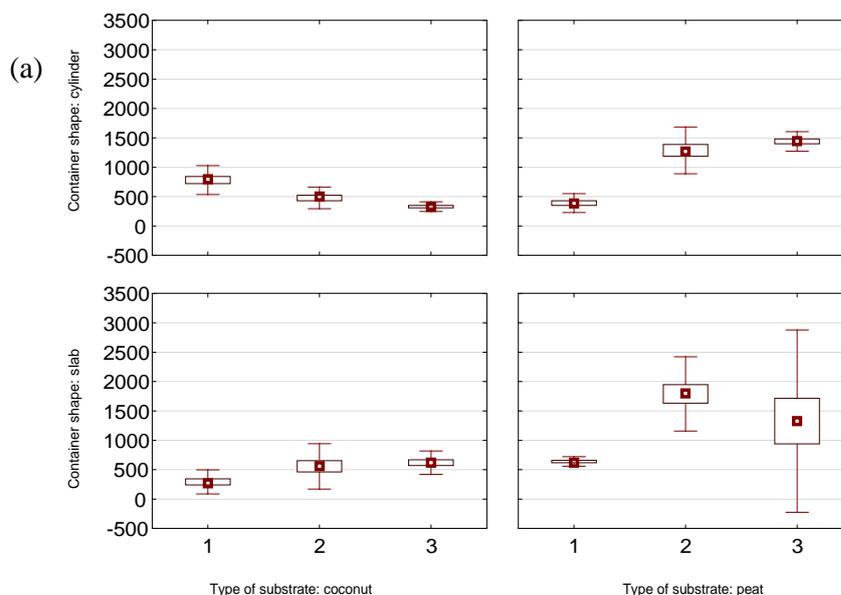
systems of tomato plants in slabs looked worse than those in cylinders. This might have also been caused by the excessive moisture in slabs (the system root is at the bottom part of a slab instead of being inside it). None of the examined factors significantly influenced the number of root tips and the root diameter. The substrate type had a significant effect on root parameters (Figure 4(c)). The better root growth on the peat substrate was caused by the higher organic and mineral composition of the peat in comparison with the coconut substrate.

3.3. Microbiological Analysis of the Rhizosphere Substrates

Microbial counts depend on the environmental conditions, which include the type of growth substrate, moisture content, season of the year, the content of organic matter, temperature, crop species, and pH [8] [9]. The applied optimal conditions for the development of tomato (pH of nutrient solution at 5.5) most probably had an advantageous effect on the growth of the analyzed groups of microorganisms and potential fluctuations in pH of substrates during culture contributed to the stronger development of individual groups. The population size of soil microorganisms is one of the parameters indicating microbiological activity of the colonized environment. Even the cultivar and its development phase determine the species composition and population size of microorganisms [10].

Much larger populations of bacteria and fungi were detected/recorded in the rhizosphere of tomato plants growing in the peat substrate than in the coconut substrate. This was related to the higher organic and mineral composition of the peat in comparison with the coconut substrate. The amount of organic matter in peat was at a level of 76.2%, whereas coconut contained 55.2% of organic matter. Similarly, in the studies on the use of different organic substrates in the cultivation of tomato, Kleiber *et al.* [11] observed almost twice as many isolated bacteria in peat than in coconut fiber. The fungal population of the rhizosphere was unaffected by the container shape, but strongly depended on the thermal factor. The total number of fungi was depended on the container type and the growing substrate. However, the total number of bacteria was going up with increasing heating level, both in the peat and coconut fiber, and in both types of containers. These results revealed that the higher energy supply to the plants (2nd and 3rd heating levels) had a positive influence on bacterial multiplication and development, whereas those energy levels decreased the fungal formation and growth in the rhizosphere. Previous studies by Shadt *et al.* [12] on the seasonal growth dynamics of fungi and bacteria suggest that in comparison with bacteria the metabolism of fungi is greater at lower temperatures. Pietikäinen *et al.* [13] also observed an increase in the ratio of bacterial to fungal growth rate at higher temperatures. The optimum temperature for growth of the most fungi varied between 20°C - 25°C [14]. In our studies, the most fungi were also found in the soil heated to approximately 20°C (Figure 6(b)).

The total numbers of bacteria and fungi in the rhizosphere samples were significantly higher in the peat substrate than in the coconut substrate (see Figures 5(a) and (b)). It might be caused by the different values of pH of substrates used in this experiment (pH of peat was measured at 4.9 and pH of coconut fibre - 6.7). The samples of coconut rhizosphere soil contained much more beneficial bacteria producing spores in regard to the peat medium (Figure 5(c)). The total number of bacteria was going up with increasing soil temperature for samples taken from the coconut slabs, peat slabs and peat cylinders. It was only decreasing for coconut cylinders (Figure 5(a)). No significant impact of the container shape on the fungal and bacterial populations was recorded.



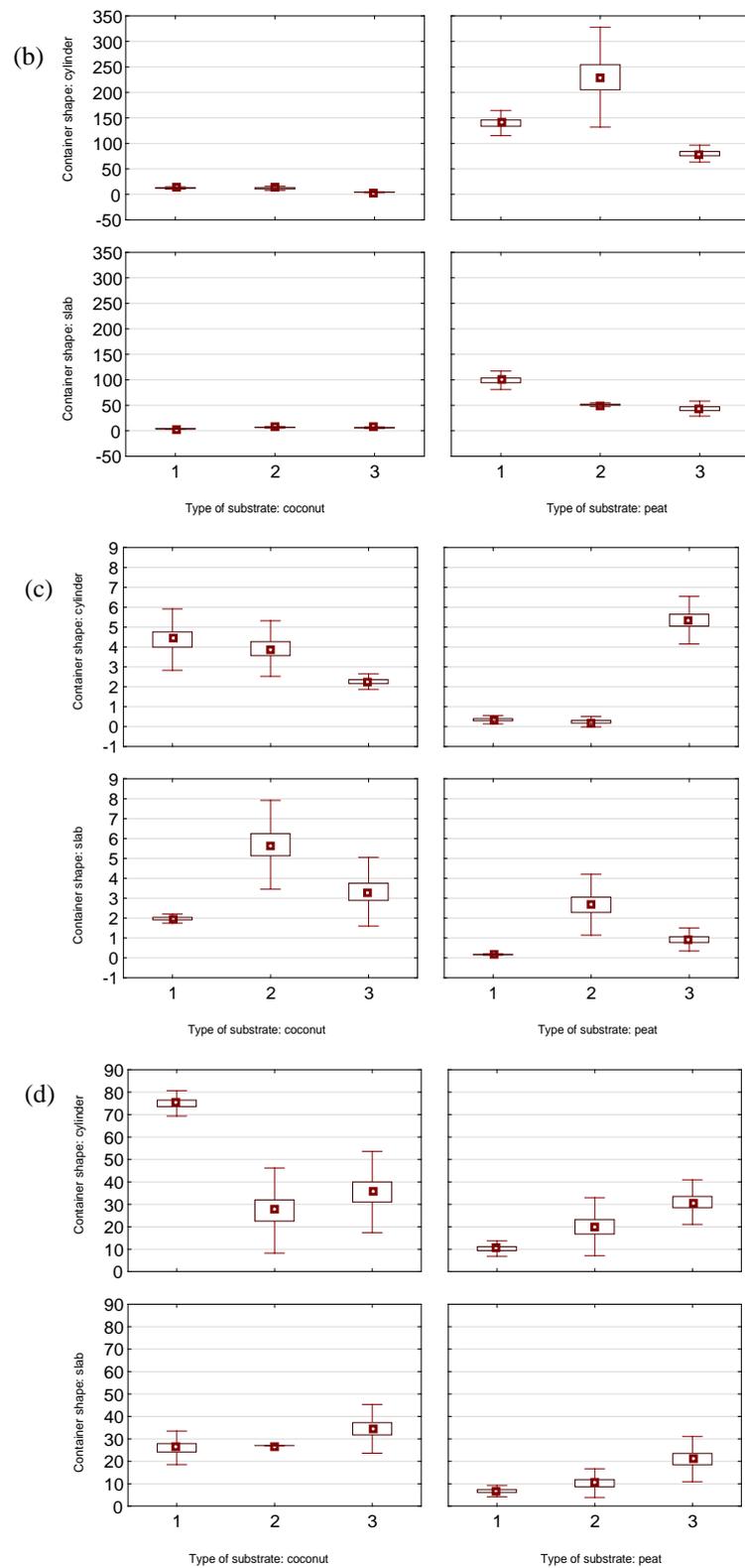


Figure 5. Expected marginal means of microorganisms (bacteria and fungi) (CFU $\times 10^3$ per g⁻¹ of fresh soil) from May rhizosphere soils at different heating levels: (a) total number of bacteria (b) total number of fungi, (c) number of bacteria producing spores and (d) actinomycetes.

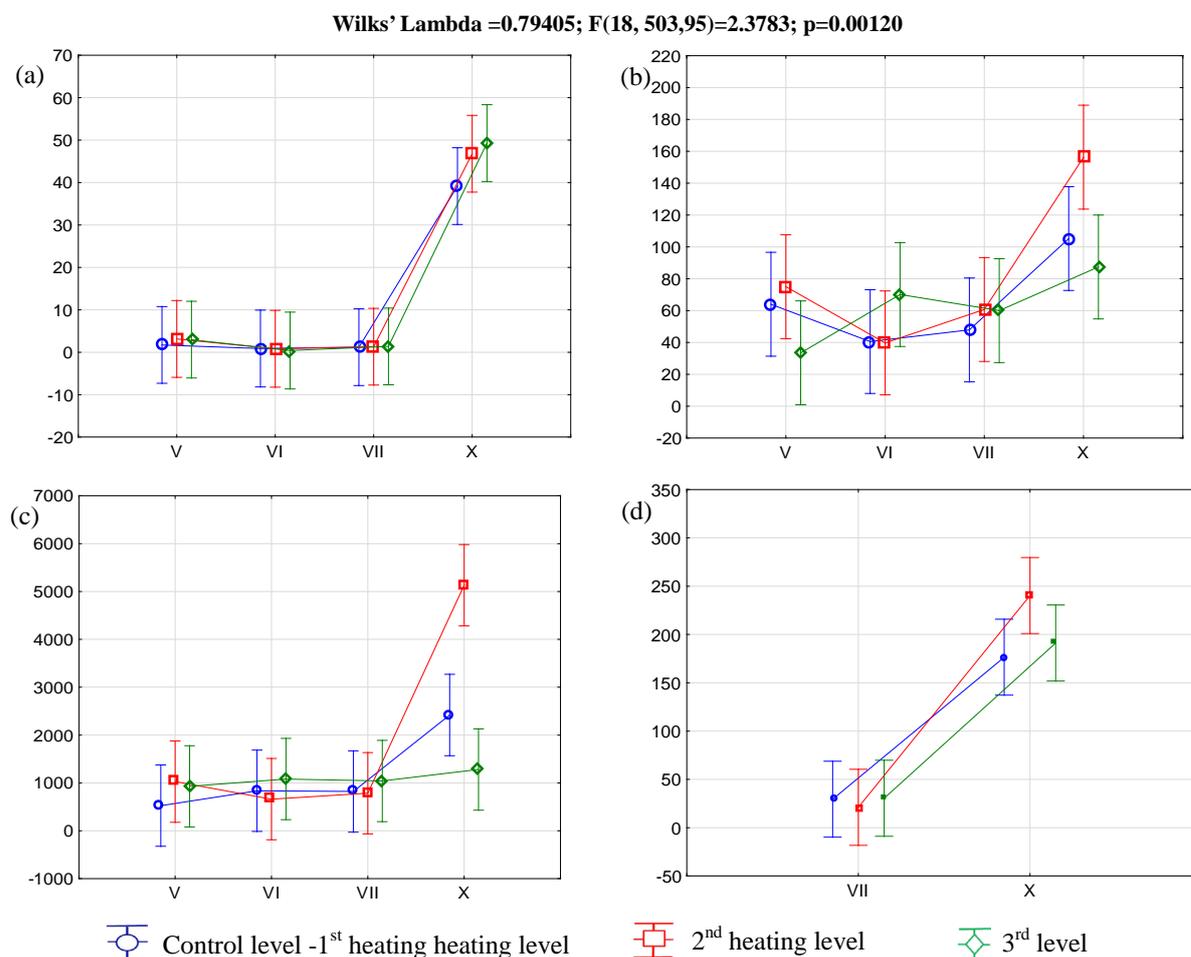


Figure 6. Expected marginal means of microorganisms (bacteria and fungi) ($\text{CFU} \times 10^6$ per g^{-1} of fresh soil) from rhizosphere soils at different heating levels during different months (or plant development phases): (a) total number of bacteria (b) total number of fungi, (c) number of bacteria producing spores, (d) actinomycetes; V—May, VI—June, VII—July, X—October; decomposition of effective hypotheses; vertical columns mean 0.95 confidence intervals (independent factors: heating level and time of soil samples collection).

The number of actinomycetes was increasing with increasing soil temperature (**Figure 5(d)**) with the exception of the plant roots/rhizosphere in the coconut cylinders. These results correspond well with those of Goodfellow and Williams [15], who observed the most efficient growth of Actinomycetes at 25°C - 30°C .

An increased temperature (reaching up even to 40°C) and an insufficient moisture content, which is frequently observed in the hot summer months (VI - VII) in the greenhouse had a significant effect on the unchangeable and similar number of microorganisms inhabiting the substrates. These high temperatures in the greenhouse slightly reduced the activity of microorganisms in peat and coconut substrates (it could be caused by inactivation of some enzymes). Stapleton and De Vay [16] and Membre *et al.* [17] observed a decrease in the number of isolated microorganisms in substrates heated to about 45°C .

This effect was visible in the unchanging population of bacteria and fungi (**Figure 6**). Lower temperatures in the autumn months (IX - X) (giving more favorable conditions for microorganisms development) could have lead to increase of microbial activity. It was associated with an increase in their population numbers (for example **Figure 6(d)**). The impact of external temperature on the population of microorganisms were clearly visible in October samples where the heat visibly influenced the number of bacteria and fungi. The highest populations of bacteria, bacteria producing spores, actinomycetes and fungi were noticed at the second heating level for October rhizosphere samples and the lowest—at the third heating level. This result suggests that in October the second heating level was the most optimal for the development of these groups of microorganisms.

3.4. Results of Nematological Analysis of Soil Samples

Soil samples for nematological analysis were taken at the end of May 2012. Amongst the known trophic groups of nematodes in a tomato cultivar only individuals belonging to the groups of bacterivorous, fungivorous and predatory nematodes were found. There were found mostly individuals belonging to the families feeding on bacteria in slab cultivations (see results in **Table 1**). The individuals coming from the Aphelenchoididae family (*Aphelenchoides* sp) were the only representatives of nematodes feeding on fungi. Besides fungivorous nematodes from the Aphelenchoididae family, individuals from the Tylenchidae family were also recorded in the rhizosphere of plants growing in peat cylinders. Moreover, predatory nematodes deriving from the family Aphelenchoididae (*Seinura* sp) appeared in this cultivation type as well (**Figure 7**), but not a lot.

4. Conclusions

The study has shown that heating the substrates limited the root growth of tomato plants. Root morphological parameters of control plants were significantly higher than the parameters of plant roots growing on heated soil. Generally, all plants at the third heating level gave much higher first weekly crops than the ones at the lower heating level, but then there were no significant differences in the total yield for all experimental plants. Plants growing in peat slabs at the third heating level gave the highest first weekly crop what can be very important from the producer point of view.

On the base of the obtained results the below conclusions are stated:

The numbers of bacteria and fungi were significantly higher in the peat substrate than in a coconut fibre.

The population of bacteria was increasing with an increase in the substrate temperature, apart from the coconut cylinders.

The number of bacteria in a coconut substrate hasn't changed significantly with an increased substrate temperature.

The container type had no significant effect on the populations of bacteria and fungi.

Total fungi decreased with increasing peat temperature.

Table 1. Nematode occurrence in the rhizosphere of tomato plants versus the soil type and the heating level.

Containers with substrate types	Heating level	Family		
		bacterivorous	fungivorous	Predatory
Slabs with coconut fibre	3	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Cephalobidae	-	-
	2	Rhabditidae, Neodiplogasteridae, Diplogasteridae	-	-
	1—Control	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Cephalobidae	-	-
Slabs with peat	3	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Cephalobidae	Aphelenchoididae	-
	2	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Cephalobidae	-	-
	1—Control	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Cephalobidae	Aphelenchoididae	-
Cylinders with coconut fibre	3	Rhabditidae, Neodiplogasteridae	-	-
	2	Rhabditidae, Neodiplogasteridae	-	-
	1—Control	Rhabditidae, Neodiplogasteridae, Cephalobidae	Aphelenchoididae	Aphelenchoididae
Cylinders with peat	3	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Cephalobidae	Aphelenchoididae Tylenchidae	Aphelenchoididae
	2	Rhabditidae, Neodiplogasteridae, Diplogasteridae, Alaimidae	Aphelenchoididae	Aphelenchoididae
	1—Control	Rhabditidae, Neodiplogasteridae, Cephalobidae	Aphelenchoididae Tylenchidae	-

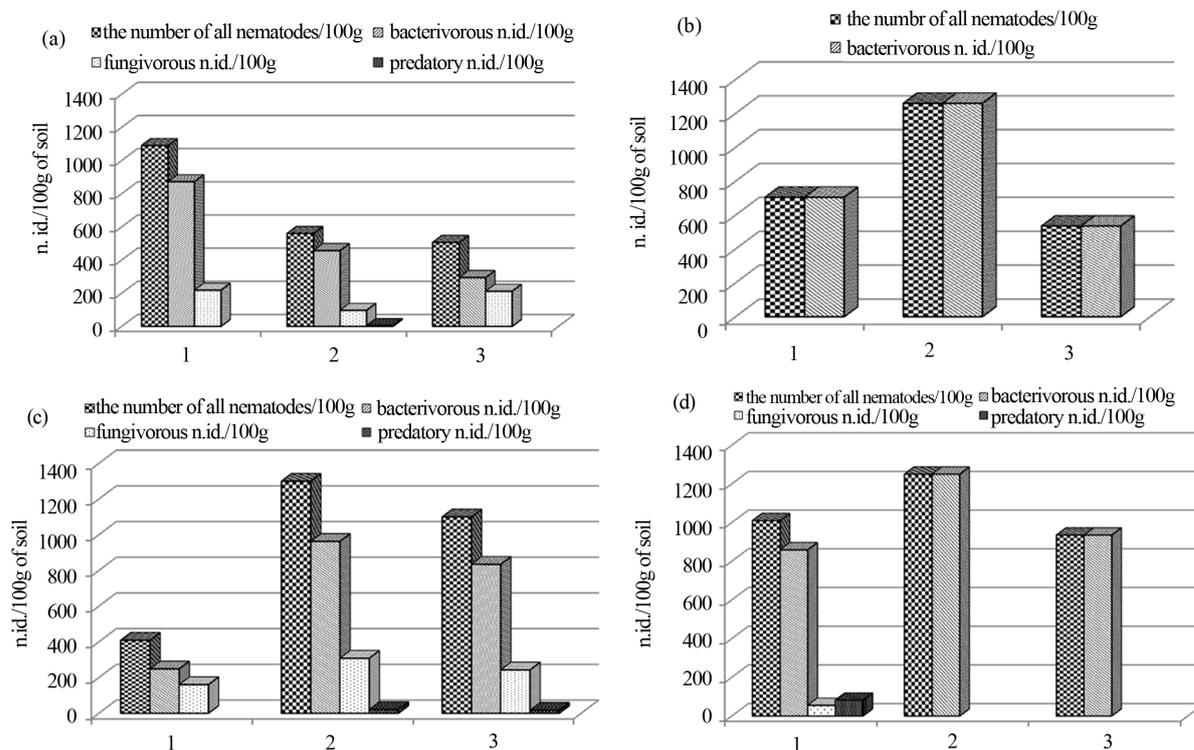


Figure 7. The number of individuals (per 100g of soil) and groups of nematodes: in slabs filled with peat (a) and coconut fibre (b); in cylinders filled with peat (c) and coconut fibre (d) against the heating level. 1—control level, 2—second heating level, 3—third heating level.

A much higher number of beneficial bacteria producing spores were noted in the coconut substrate.

Summer months limited the development of the population of bacteria and fungi.

The total number of bacteria and fungi was highest at three energy levels for samples collected in October.

The largest/biggest number of nematodes was recorded at the second energy level, with the exception of cultivation carried out in a peat substrate in slabs, where most of nematodes were found at the control level.

The interaction of the container type and substrate type significantly influenced the morphology of the root system.

Plants growing in the cylinders on peat substrates had the highest root growth characteristics.

Further investigations on the number of beneficial microorganisms depending on the heating level and the type of the growth substrate will be continued to answer which heating level is the most favorable for the development of these groups of microorganisms, e.g. types of bacteria (e.g. *Azotobacter*, *Pseudomonas fluorescens*) and fungi in the rhizosphere.

Acknowledgements

The work has been supported by the grant from the EU Regional Development Fund through the Polish Innovation Economy Operational Program, contract No. UDA-POIG. 01.03.01-10-115/09.

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