Identification of microbial infections in Greek geothermal greenhouses tomato cultivation

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Abstract. The main object of this work is the identification of possible microbial infections in tomato cultivation in geothermal greenhouses. The hydroponical system, is a "close type" (for fertilization and irrigation water reuse), while at the same time exploiting rainwater collected from the gutters of greenhouses. The purpose of this experimental work is to identify the detection of pathogenic microorganisms (total microbial flora, E. Coli, coliforms, Enterococci and Cl. Perfrigen) in tomato fruits and also to fertigation water used. The main question of this research is about the possibility of contamination of tomato fruits with bacteria. From the microbiological analyses performed it was revealed that populations of E. coli were not detected, while the populations of coliforms identified were within the permissible limits of intestinal bacteria (coliforms <10000), which can be derived and detected in tomato from natural inputs such as of water, soil and insects.

1 Introduction

Geothermal energy has been used since ancient times for therapeutic uses, bathing and water heating. There are many testimonies in China, Japan and America [1,2] that prove the use of natural hot fluids by the ancient peoples. It is worth noting that the Etruscans and the Romans in particular used hot springs to heat their homes [3]. The Homeric epics as well as the writings of Herodotus, Pausanias and Aristotle testify to the importance of hot springs for the ancient Greeks. The use of hot spring water for thermal and religious purposes has been reflected in many pots that have been found until today. Galinos in the 2nd century A.D. produced fruits and vegetables out of season because he created the first greenhouses. Greenhouse energy requirements are directly related to the prevailing climatic conditions, while their heating costs represent up to 35-40% of operating costs. The geothermal energy use, take advantage of the constant underground water temperature, up to 150m deep, can cover the energy requirements of the greenhouses. The transfer of the geothermal fluid (water) can be done with insulated pipes, and its transfer can be done either directly inside the greenhouse with plastic pipes or indirectly with the help of a heat exchanger. The use of geothermal energy can offer many advantages and economic growth, since an average greenhouse can save a very large part of its operating costs from fuel, which can even exceed 75%. Depending on the temperature of the subsoil, the geothermal fields are divided into the following categories: high enthalpy when the temperature is greater than 150 °C, medium enthalpy when the heat is 90-150 °C, low enthalpy with temperatures of 25-90 °C, very low enthalpy less than 25 °C and 0 °C. Geothermal applications are for electricity generation, district heating, desalination, industry, agricultural uses, aquaculture, spa therapy, and heating of swimming pools and treatment units. Geothermal energy is generally considered environmentally friendly and has small environmental footprint. Geothermal is satisfactory for heating and cooling and can benefit small households. The production of geothermal energy has no dependence on fossil fuels and therefore has no price fluctuations. The disadvantages of geothermal are mainly focused on the fact that: Geothermal power plants can in extreme cases cause earthquakes. Geothermal power plants must have a very specific location. Geothermal sources have the possibility of being exhausted. High investments are also required for the cost of the geothermal system. Large areas of land are also required for the installation of the geothermal system. The environmental impacts of geothermal energy are related to pollution of surface water and atmosphere, the formation of deposits on equipment, noise from the operation of geothermal facilities, corrosion of metal transmission pipes, disturbance of flora and fauna from drilling, thermal pollution from the disposal of geothermal fluids [4].

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Scientists [5-8] have found that the roots of plants mainly of lettuce absorb through their roots with irrigation water the E. coli species which is transferred to leaves and stems.

2 Materials and methods

2.1 Materials

5 cm diameter petri dishes, sterile filters, sterile pipette tips, sterile beakers.

2.2 Chemical reagents

Iron Sulfate, Sodium sulphite, Sodium hydroxide (NaOH 1 N).

2.3 Nutrient substrates

Yeast extract agar, Slanetz and Bartley medium, Chromogenic Coliform agar (CCA).

2.4 Scientific instruments

Water bath, water filter, incubators, burner, shaker, colony counter, pH meter, autoclave.

2.5 Points and method of sampling, frequency

The sampling was done at the University of Thessaly, in the geothermal greenhouses. Their operation is based on the use of "closed" hydroponic cultivation systems for both fertilization and irrigation of the tomato. Sampling was done on five different dates from April to June 2022. Sampling points included the inlet of the dripper which gives all nutrients to the plant (Figure 1), the exit from the dropper (Figure 2) which supplies the nutrients to the plant, the skin and the stem of the tomato. Water samples were collected in a sterile 500 ml bottle.

2.6. Microbiological analyses

The microbiological analyzes carried out on all the samples of both the irrigation water [9,10,11] and the skin of the tomato and its stem concerned the microbiological analyse of total microbiological flora [12], E. coli, coliforms [13], Cl. Perfrigen [14], Enterococci [15].

Table 1. Substrates and bacteria isolated

Type of bacteria	*Nutrient substrates*
Total microbiological flora	Yeast extract agar
Cl. Perfrigen	Sulfite iron agar
Enterococci	Slanetz and Bartley medium
E. Coli	Chromogenic Coliform agar
Coliforms	Chromogenic Coliform agar

Fruit (Figure 3) and tomato stem (Figure 4) samples were collected from different parts of the greenhouse to get a better overall picture of the microbial load and placed in a sterile bag.

Fig. 1. Sample from the inlet dropper.

Fig. 2. Sample from the outlet dropper

Fig. 3. Placing tomato sample in sterile bags.

Then the samples were transferred to the laboratory and all the necessary procedures of the protocols were followed.

3 Results

Fig. 4. Tomato stem sample

The following images (Figure 5-12) show the results from the microbiological analyzes of inlet and outlet water.

3.1. Irrigation water microbiological analyses

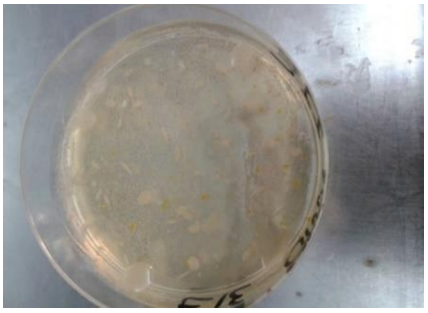

Fig. 5. Development and counting of Total microbiological flora 22°C.

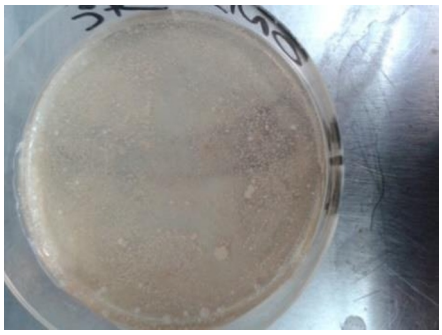

Fig. 6. Development and counting of Total microbiological flora 37°C

3.2. Inlet water microbiological analyzes

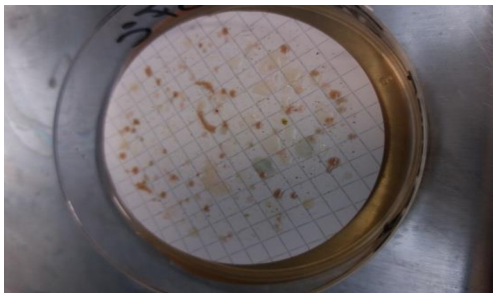

Fig. 7. Development and counting of Enterococci bacteria

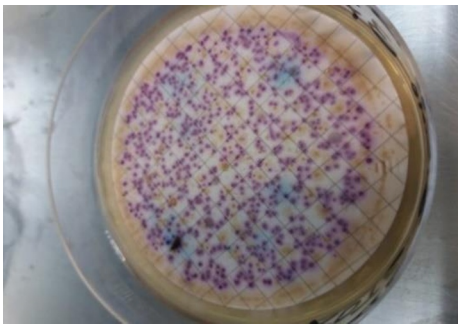

Fig. 8. Development and counting of E. Coli & Coliforms bacteria

3.3. Outlet water microbiological analyses

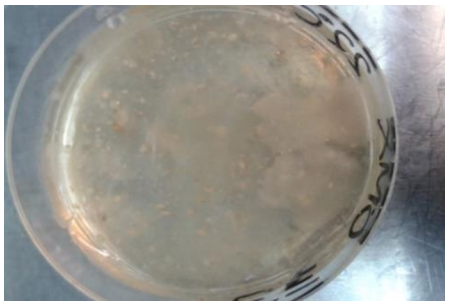

Fig. 9. Development and counting of Total microbiological flora 22°

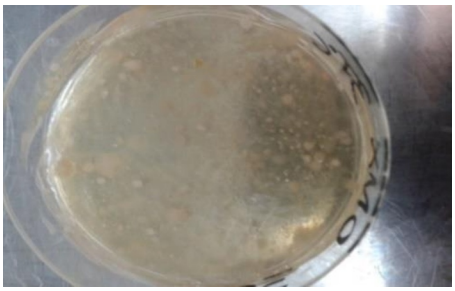

Fig. 10. Development and counting of Total microbiological flora 37°C

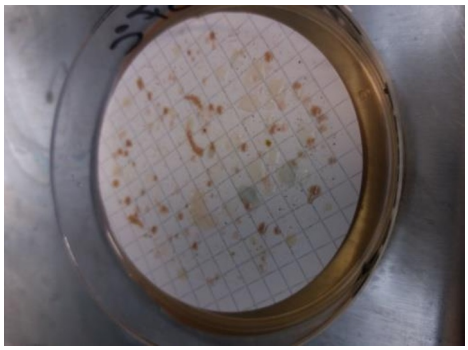

Fig. 11. Development and counting of Enterococci bacteria.

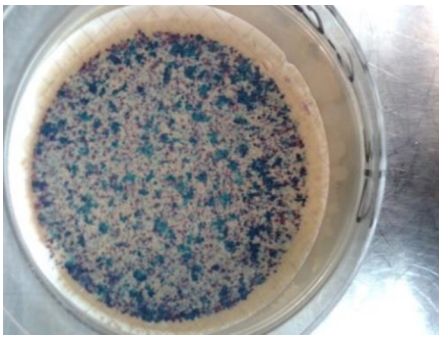

Fig. 12. Development and counting of E. Coli & Coliforms bacteria

Colonies were then counted (Table 2).

Table 2. Colony count results

Type of bacteria	Entrance	Exit
Total microbiological flora 37°C	$1,1X10^3$	$1,8X10^3$
Total microbiological flora 22°C	$1,0X10^3$	$1,7X10^3$
Coliforms	$1,2X10^3$	$1,3X10^3$
E. Coli	$0,6X10^3$	$0,7X10^3$
Enterococci	$1,2X10^1$	$2,5X10^1$

3.4. Conclusions on irrigation water analyses

The number of microorganisms at the outlet appears to be much higher than at the inlet. This is explained because in addition to those present in the nutrient material of the drips, the microorganisms from the tomato growth substrate are also added.

3.5. Stem and skin tomato microbiological analyze

After collecting a sufficient amount of tomato skin and stem sample, they were placed in sterile bags and then the sample was transferred to sterile beakers containing 200 ml of MRD and placed in the shaker for 15 minutes. The microbiological analyses performed on all samples were analyse of total microbiological flora (ISO 6222), E. coli, coliforms (ISO 9308-1), Cl. Perfrigen (ISO EN 26461-2), Enterococci (ISO 7899-2) are shown in the Figures 13-18.

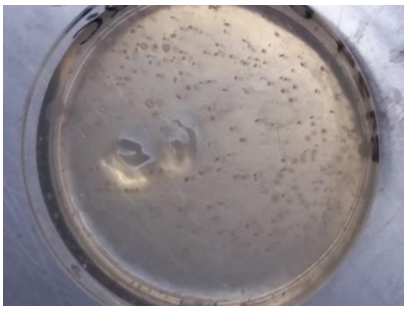

Fig. 13. Total microbiological flora 37°C of stem.

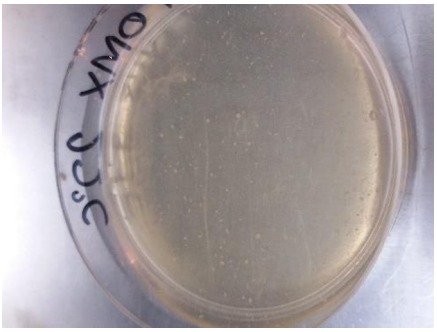

Fig. 14. Total microbiological flora 22°C of stem.

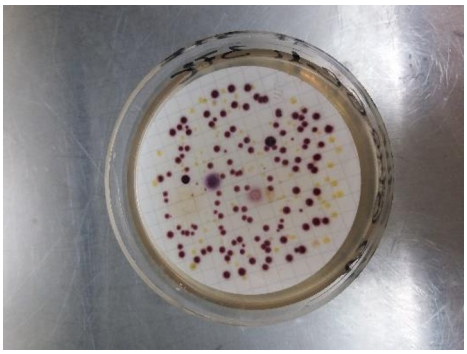

Fig. 15. Stem *Coliforms* bacteria.

Fig. 16. Total microbiological flora 37°C of skin.

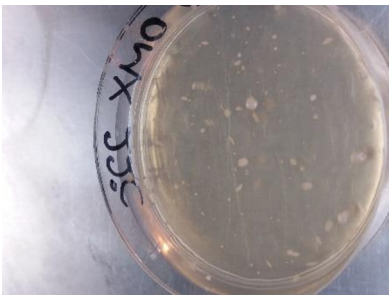

Fig. 17. Total microbiological flora 22°C of skin.

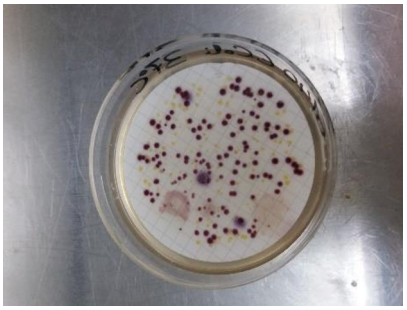

Fig. 18. Skin *Coliforms* bacteria.

Table 3. Colony count results

Type of bacteria	Stem	Skin
Total microbiological flora 37°C	$1,6X10^2$	$1,4X10^2$
Total microbiological flora 22°C	$1,2X10^2$	$3X10^1$
Coliforms	$0,9X10^2$	$0,75X10^2$
E. Coli	Absence	Absence

4 Conclusions

Based on the legislation of the EC committee [16] when all the values of the count of E. Coli and Coliforms are below 102 cfu in five consecutive samplings then the food is judged as suitable for eating. In the present study, the pathogenic bacteria variations of E. Coli and Coliforms during the greenhouse tomato growth were examined. The investigation of the presence of E. Coli and Coliforms bacteria was carried out in skin and stem samples of the tomato fruit. In all cases no populations of E. coli were detected, while the populations of Coliforms detected were within the permissible limits (Coliforms <103) of intestinal bacteria. Intestinal bacteria can originate and be detected in tomato fruit from natural contamination such as water, soil and other causes. In particular, typical Enterobacteriaceae genera such as Citrobacter, Enterobacter, Hafnia, Serratia and Klebsiella have been reported in irrigation water and soil and have been characterized as genera of environmental origin [17]. The bacterium E. coli was not isolated in the present study which proves that the irrigation water does not have faecal contamination bacteria. Undoubtedly, in the present study, there are no waterborne pathogenic bacteria in the skin of the tomato which may cause colonization of the plant tissues with additional risks. The absorption phenomenon of E. coli species has also been reported in hydroponic cultivation of tomato [18] where E. coli species is transported from the roots with the irrigation water and settles peripherally in the network of trichomes on leaves and on tomato stems of the plant. All of the above reinforce the results of the present study, that is, the absence of water bacteria with fecal substances such as E. coli, in the greenhouses of the University of Thessaly, at Gaiopolis campus, Larissa, which may cause colonization of tomato tissues through irrigation.

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