

## THE EFFECT OF SILICON AND CALCIUM ADDITIVES ON THE GROWTH OF SELECTED GROUPS OF MICROORGANISMS IN SUBSTRATE USED IN SOILLESS CULTIVATION OF STRAWBERRIES

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### ABSTRACT

The aim of the study was to evaluate the impact of silicon (Si) and calcium (Ca) added to the substrate (perlite or its mixture with peat) used in soilless strawberry cultivation on the number of different groups of microorganisms in the substrate. Research was conducted on a farm located in southern Poland in which soilless cultivation of strawberries in gutters, under covers, with an irrigation system was carried out. The microbiological analyzes were performed by serial dilution method. The analyzes included determination of the total number of bacteria, actinobacteria, fungi and aerobic atmospheric nitrogen assimilators of the *Azotobacter* genus. In this work, we showed that the concentration of microorganisms associated with the cultivation substrate may be influenced by the presence of silicon and calcium added to the composition of the substrate. Correlation analysis showed that the addition of Si + Ca to the substrate affects increase in the total number of bacteria in the substrate. The obtained results confirm that the cultivation substrate can be modified in such a way that it is more conducive to the multiplication and survival of bacteria associated with the substrate.

**Key words:** strawberry, substrate, microorganisms, silicon, calcium

### INTRODUCTION

In recent years, more and more often attention is paid to the search for new factors improving the process of nutrient uptake by plants from the soil with simultaneous improvement of their chemical, biological and physical properties. Physical properties as well as the level of nutrients in cultivation media influence plant growth and health [Vandecasteele et al. 2018]. Twelve basic elements play an important role in plant growth, including macro- and micronutrients (N, P, K, Na, Mg, S, Ca, Mn, Zn, B, Cu, Mo). However, other elements, including silicon [Niewiadomska 2013, Górski et al. 2017], may indirectly stimulate plant growth, contributing to the development of the roots of crop plants. The results of many studies indicate

that silicon affects the growth, development and resistance of plants to various stress factors, both biotic and abiotic, e.g. increased resistance to disease, drought, salinity, oxidative stress [Balakhnina and Borkowska 2013]. The beneficial effect of silicon fertilization was also found in the case of the growth of the root system of strawberries [Borkowski et al. 2014]. Over the past two decades, world strawberry production has increased by 55%. In recent years, an important issue in the cultivation of strawberries, as well as other crops, is the search for new cultivation technologies. To increase profits, strawberries are grown both in soil and in soilless media [De Tender et al. 2021]. The possibility of their soilless cultivation, in gutters under covers

and with the irrigation system gives hope for further increase in their production. The term „soilless culture” generally refers to any method of growing plants without using soil as a medium for roots [Savvas and Gruda 2018].

The main advantage of soilless cultivation is the independence of cultivation from soil, which – as a natural substrate – is heterogeneous, contains pathogens, tends to degrade in monoculture systems and may be infertile, alkaline or acidic. Soil is an environment whose physical, chemical and biological properties may change during the agricultural production process due to various abiotic and biotic factors (e.g. fertilization). These changes not always have a positive effect on the condition of the soil and its agrotechnical usefulness. In case of modern soilless cultivation systems provide all the necessary nutrients for plants [Breza-Boruta 2013, Savvas and Gruda 2018]. Soilless cultivation of strawberries consists in providing plants with the right amount of nutrients, which not only positively affect plant growth and health, but also positively affect the biological properties of the substrate [De Tender et al. 2016, Vandecasteele et al. 2018]. The selection of a soilless cultivation substrate for plant nutrition and growth is crucial to improving the ecological sustainability of production in horticulture systems [Grunert et al. 2016]. Currently, the most popular worldwide is the production of vegetables or flowers on horticultural cultivation substrates, such as mineral wool, perlite or coconut fiber [Savvas and Gruda 2018]. Commonly available data of the presence of the microorganisms population in the cultivation substrates for use in soilless cultivation are still very limited. The results of many studies indicate that the method of cultivation and the addition of fertilizers have numerous effects in heterotrophic processes occurring in the soil, including changes in enzyme activity, respiration processes and decomposition [Sinsabaugh et al. 2005], as well as changes in the abundance and composition of soil microbiota – both bacteria [Campbell et al. 2010] as well as fungi [Frey et al. 2004]. Increase in microbial populations in cultivation substrate is a key factor affecting the rate of circulation of nutrients, production of plant growth regulating materials and their resistance or tolerance to plant pathogens [Arancon et al. 2006]. Interactions between plant roots and the microbial populations also play a major

role in plant growth and productivity [Pascual et al. 2018]. Microorganisms with plant cover determine the direction and nature of biochemical processes and lead to the creation of a balance in soil environments and protect against the invasion of pathogens [Natywa et al. 2014, Grunert et al. 2016, Chen et al. 2020, Drobek et al. 2021]. The studies showed that fertilization with microbiological preparations increased the strawberry fruit yield and improved the properties of strawberries [Sas-Paszt et al. 2020, Drobek et al. 2021]. The presence of specific beneficial microorganisms may affect the concentration of some elements and volatile substances in strawberry fruits. Plant growth promoting bacteria (PGPB) present in the soil affect the nutritional quality of strawberries by increasing the concentration of sugar and anthocyanins, and regulate the levels of pH, malic acid, volatile compounds and elements [Castellanos-Morales et al. 2010, Todeschini et al. 2018]. The aim of the study was to determine the quantitative changes in bacterial and fungal communities inhabiting the cultivation substrate with the addition of silicon (Si) and calcium (Ca) – perlite or its mixture with peat – used in soilless cultivation of strawberries.

## MATERIAL AND METHODS

The research was carried out on the experimental plantation of the Stanflex – Polski Instytut Truskawki sp. z o.o. (Stanflex – Polish Strawberry Institute, limited company) in a gutter system under roofing. The everbearing strawberry (i.e. one with repeated fruiting), was carried out in a gutter system under roofing, on a soilless substrate. The amendment of Si + Ca was prepared from natural carbonate-silica rock named opoka, consist of an average 50% CaCO<sub>3</sub> and 36% SiO<sub>2</sub> (Cucarella and Renman 2009). The silica in this rock is mainly amorphous, derives from organic detritus, mainly diatoms. Before use, the material was burned at a temperature 900°C to obtain more CaO oxide forms. The material was ground into a silt fraction (<0.05 mm). In the experiment, substrates with bags volume of 20 dm<sup>3</sup> (100 cm × 20 cm × 10 cm) were used with a volume composition of 80% expanded perlite (fraction of 2–6 mm), 20% transitional peat and the appropriate amount and crushed opoka of 0 cm<sup>3</sup>, 5 cm<sup>3</sup>, 10 cm<sup>3</sup>, 15 cm<sup>3</sup> and 20 cm<sup>3</sup>). The amount of the

optimal dose of Si + Ca amendment (10 cm<sup>3</sup>) needed to optimize the pH value of the substrate to the level of about 5.8 was determined based on the CaO content and the cation exchange capacity of the peat. The amount of Si + Ca amendment was poured in bulk. The Si + Ca amendment was used once during the preparation of the substrate according to the procedure and recipe of the Stanflex Company. The strawberries were planted in two rows of eight seedlings per one substrate. One repetition was 4 m length (4 substrates) and 32 strawberry plants. An average sample was taken from four locations, one on each substrate. At the plantation, there were cultivated: strawberry seedlings (*Fragaria × ananassa* Duch.) specimens ‘Amandine’ (AM) and ‘San Andreas’ (SA). Frigo seedlings were planted in two classes of the diameter of the root neck A (11–14 mm) and A+ (>15 mm).

In the conducted experiment, the following variants were used:

- 1 – control sample: AM (A) + 0 cm<sup>3</sup> Si + Ca,
- 2 – AM (A) + 5 cm<sup>3</sup> Si + Ca,
- 3 – AM (A) + 10 cm<sup>3</sup> Si + Ca,
- 4 – AM (A) + 15 cm<sup>3</sup> Si + Ca,
- 5 – AM (A) + 20 cm<sup>3</sup> Si + Ca,
- 6 – control sample: AM (A+) + 0 cm<sup>3</sup> Si + Ca,
- 7 – AM (A+) + 5 cm<sup>3</sup> Si + Ca,
- 8 – AM (A+) + 10 cm<sup>3</sup> Si + Ca,
- 9 – AM (A+) + 15 cm<sup>3</sup> Si + Ca,
- 10 – AM (A+) + 20 cm<sup>3</sup> Si + Ca,
- 11 – control sample: SA + 0 cm<sup>3</sup> Si + Ca,
- 12 – SA + 5 cm<sup>3</sup> Si + Ca,
- 13 – SA + 10 cm<sup>3</sup> Si + Ca,
- 14 – SA + 15 cm<sup>3</sup> Si + Ca,
- 15 – SA + 20 cm<sup>3</sup> Si + Ca,
- 16 – control sample: SA (A+) + 0 cm<sup>3</sup> Si + Ca,
- 17 – SA (A+) + 5 cm<sup>3</sup> Si + Ca,
- 18 – SA (A+) + 10 cm<sup>3</sup> Si + Ca,
- 19 – SA (A+) + 15 cm<sup>3</sup> Si + Ca,
- 20 – SA (A+) + 20 cm<sup>3</sup> Si + Ca.

During the vegetation, main fertilizers were used in the following doses (per running meter of the mat): calcium nitrate – 156.8 g, ammonium nitrate – 3.587 g, magnesium nitrate – 34.97 g, DTPA iron chelate 7% Fe – 2.130 g, monopotassium phosphate – 22.51 g, potassium nitrate – 34.26 g, magnesium sulphate – 17.70 g, 13% EDTA manganese chelate – 1.875 g, and nitric acid – 86.92 g. Substrate samples

for microbiological tests were collected from each experimental variant, in the beginning, in the middle and at the end of gutters with grown strawberries (about 500 g), two times during the growing season of plants, first flowering and first fruiting period. The value of pH was determined electrochemically (pH meter CP-505), while the electrolytic conductivity (EC) was determined via conductometry (CPC-502 conductivity meter) while maintaining the ratio of material to water was 1 : 5. The pH measurements were made before planting the plants and the fertigation medium was controlled at the level of pH 5.8 and Ec 1.2 (pH = 5.5, EC = 1.2–1.7 mS·cm<sup>-1</sup>). Microbiological analyzes were performed by serial dilution method [Pepper et al. 1995]. A series of ten-fold dilutions up to 10<sup>-6</sup> were made from each substrate sample. Analyzes included determination of the total number of bacteria – on the nutrient agar – MPA (2 days, 37°C) [Atlas and Parks 1997], actinobacteria – on Gausse’s medium (7 days, 28°C) [Atlas and Parks 1997], fungi – on agar with malt extract – MEA; Oxoid Ltd., Basingstoke, Hampshire, Great Britain (5 days, 28°C) and oxygen atmospheric nitrogen assimilators of the genus *Azotobacter* – on the Ashby’s medium (5 days, 28°C) [Atlas and Parks 1997]. All determinations were carried out in three replications. The number of CFUs (colony forming units) of microorganisms was determined by the culturing method, converting the result of the determination to one gram of substrate dry matter (CFU·g<sup>-1</sup> d.m. of substrate). Dry mass of the substrate was determined using a moisture analyzer (MB35 model, OHAUS, USA). From each of the experimental variants, along with the collection of the substrate samples, the effluent samples were taken from the cultivated gutters. The microbiological tests included the determination of the same groups of microorganisms as in the case of studied cultivation substrate. The microbiological media used for analyzes and the principles of analysis of microorganisms were presented in this work, in the methodology for cultivation substrate testing. The results were converted into 1 cm<sup>3</sup> of the effluent and presented in the form of CFU.

#### Statistical procedures

Statistica 13 (StatSoft, Inc., Tulsa, OK, USA) was used for statistical data analysis. The analysis of variance (one-way and two-way ANOVA) was calculated

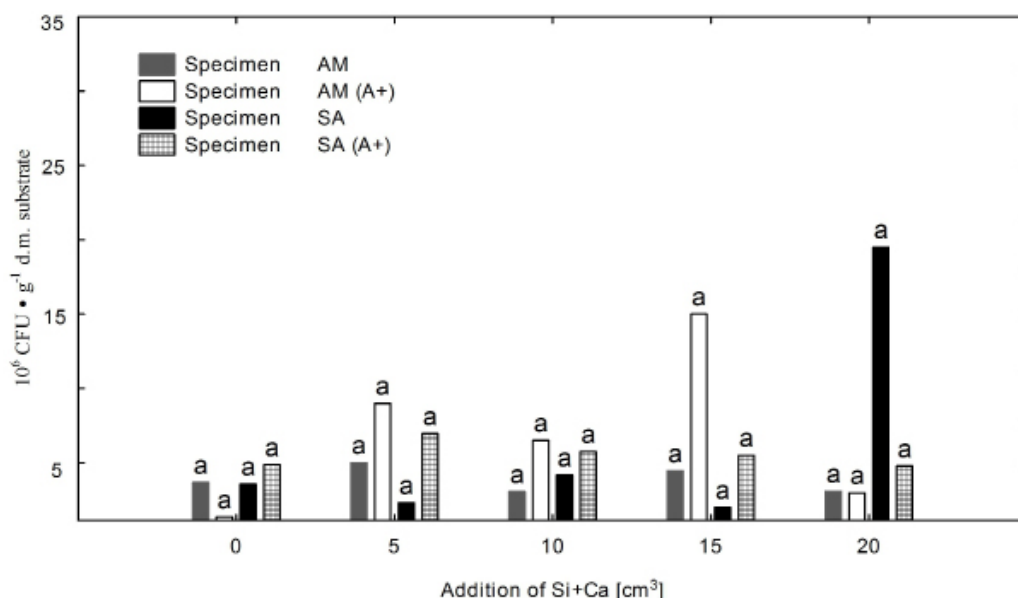
and the significance of differences between means was verified by Tukey's test ( $\alpha = 0.05$ ). The assessment of the effect of pH and silicon (Si) content in the substrate on the observed numbers of the studied groups of microorganisms and the correlation between the occurrence of the studied groups of microorganisms in the substrate and their presence in the effluent were assessed using Pearson's correlation coefficient, assuming statistically significant values for  $p < 0.05$ .

## RESULTS AND DISCUSSION

Plants take up Si into their root from the soil in the plant-available forms [Raven 2001]. Silicon in the form of silicic acid [ $\text{Si}(\text{OH})_4$ ] or mono silicic acid [ $\text{H}_4\text{SiO}_4$ ] can cross the plasma membrane of the root at physiological pH. Silicon comes from opoka rocks is amorphous and much easier available than mineral silica comes from minerals quartz or opal. In this study was assumed that silica could be available and effectively taken from soil or substrate by the roots system [Jayawardana and al. 2014, Kim et al. 2014], especially amorphous silicon can be effectively taken by the root system [Raven 2001]. Some authors give

details about the role of Si in both plants growth and development, and its efficiency in providing tolerance against various environmental stresses [Zargar et al. 2010, 2012, 2019].

Data of the dynamics of growth of selected groups of microorganisms present in the substrate with the addition of silicon and calcium, used in soilless gutter cultivation of various strawberry specimens are presented in Figures 1–4. Based on the obtained results, quantitative differentiation of the studied physiological groups of microorganisms was found. The range of average total number of bacterial and fungal microorganisms in the analyzed samples of the substrate was: in the case of bacteria from  $9.75 \times 10^5 \text{ CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$  up to  $300.00 \times \text{CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$ , for actinobacteria from  $0.15 \times 10^4 \text{ CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$  up to  $75.00 \times \text{CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$ , for *Azotobacter* bacteria from  $0.05 \times 10^3 \text{ CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$  up to  $4.5 \times 10^3 \text{ CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$ , and in the case of fungi from  $0.10 \times 10^4 \text{ CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$  up to  $18.00 \times 10^4 \text{ CFU} \cdot \text{g}^{-1} \text{ d.m. substrate}$ . In the case of total number bacteria, the biggest differences for their average values were observed between the substrate with addition of  $20 \text{ cm}^3$  of Si + Ca under SA strawberry-



**Fig. 1.** The effect of applied Si + Ca additives on the average total number of bacteria in cultivation substrate from different strawberry specimens cultivation

**Table 1.** pH values of the tested substrate for strawberry cultivation

Strawberry variety and addition of (Si + Ca) [ml]	pH (range) min–max
AM (0)	4.49–5.07
AM (5)	4.61–5.24
AM (10)	4.54–5.08
AM (15)	4.77–5.08
AM (20)	4.96–5.31
AM A+ (0)	4.83–5.30
AM A+ (5)	5.03–5.88
AM A+ (10)	5.26–5.68
AM A+ (15)	5.22–5.48
AM A+ (20)	5.31–5.43
SA (0)	5.28–5.51
SA (5)	5.30–5.81
SA (10)	4.91–5.64
SA (15)	5.07–5.42
SA (20)	5.19–5.26
SA A+ (0)	5.39–5.82
SA A+ (5)	5.48–5.66
SA A+ (10)	5.22–5.35
SA A+ (15)	5.40–5.56
SA A+ (20)	5.23–5.49

ry specimen relative to the substrate without additives under AM (A+) specimen (respectively  $195.00 \times 10^5$  CFU·g<sup>-1</sup> d.m. substrate and  $13.50 \times 10^5$  CFU·g<sup>-1</sup> d.m. substrate), however, these differences were not statistically significant (Tukey's test,  $p > 0.05$ ). Usually the average number of bacteria in the tested experimental variants was higher under the AM (A+) strawberry specimen (Fig. 1).

Based on the obtained results, two-way analysis of the ANOVA variance showed no significant effect of the addition of Si + Ca to the substrate and the specimens of cultivated strawberries on the number of selected groups of microorganisms (Tukey's test,  $p > 0.05$ ). The highest total number of bacteria in cultivation substrate with addition of 20 cm<sup>3</sup> of Si + Ca is probably related to a greater Ca supply to the substrate and more favorable (pH) of the cultivation substrate

environment for growth of microorganisms in this system (Tab. 1).

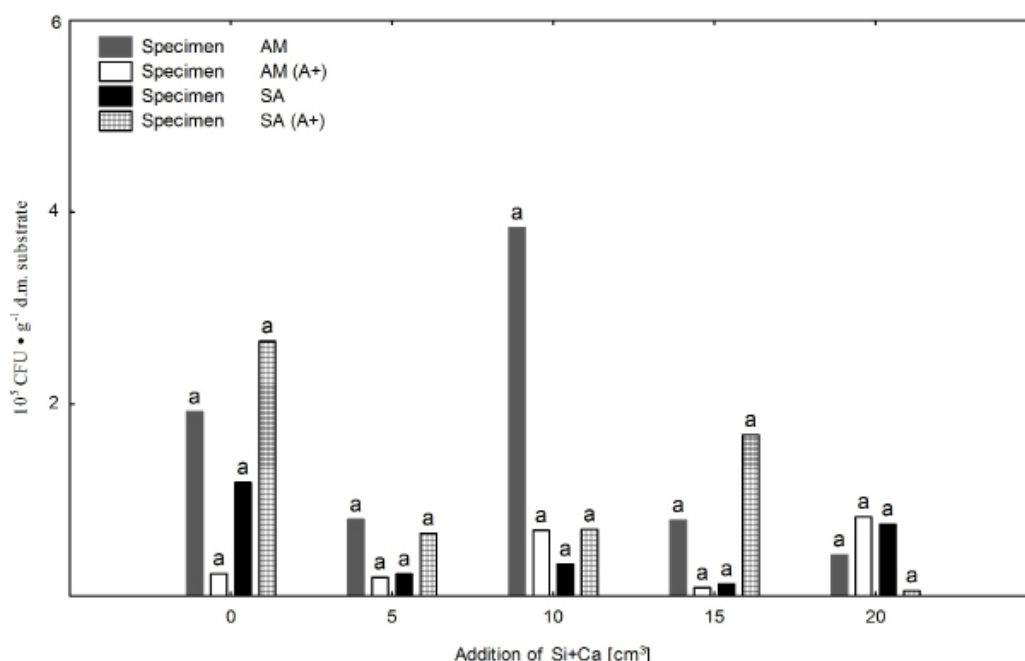
The pH and humidity are the main factors influencing the bacterial populations present in the substrates [Grunert et al. 2016]. It should be emphasized that the optimal pH value facilitates microbial growth and improves soil quality [Karunakaran et al. 2013]. Similar observations regarding the influence of pH of the soil environment on the growth of microorganisms have been received by Martyniuk et al. [2007]. Under certain conditions, silicon compounds also support and increase bacterial growth. In general, the solubility of silicon increases with the pH value of the soil and has the highest value in the alkaline reaction range. Various silicon sources do not affect largely on the pH of the soil, thereby maintaining the optimum pH for growth of bacteria [Karunakaran et al. 2013, Umama-

heswari et al. 2016, Vasanthi et al. 2018]. Karunakaran et al. [2013] observed in all soils incorporated by silica (all silica-incorporated, with the exception of sodium silicate) increased growth of the bacterial population. Bacteria use silica and reduce content of that compound in soil [Wainwright et al. 2003] (Fig. 1).

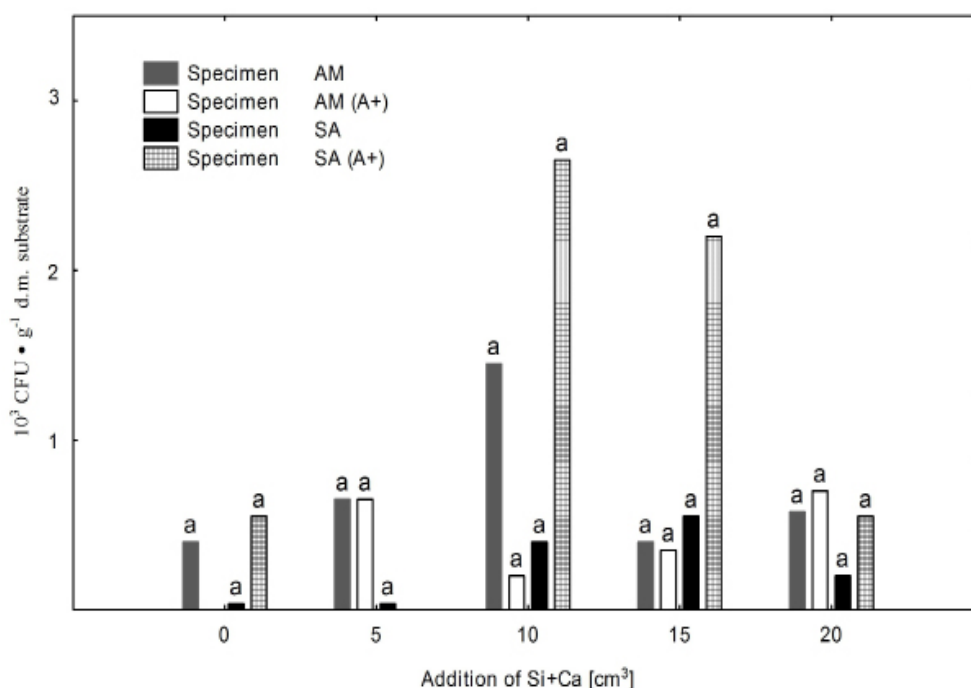
Correlation analysis showed that the addition of Si + Ca to the substrate affects increase in the total number of bacteria in the substrate (Pearson's correlation index: at  $p < 0.05$ ). Chobotar'ov et al. [2010] showed that silica stimulates bacterial growth at various concentrations as a result of the interaction that occurs with bacterial cells. Silicon can also stimulate the vegetative growth of strawberry and affect the composition and yield of fruit [Todeschini et al. 2018, Soppelsa et al. 2019]. In the case of actinobacteria, it was found that the highest average number of actinobacteria was recorded for the variant in which 10 cm<sup>3</sup> of Si + Ca was applied under cultivation of the AM specimen ( $38.39 \times 10^5$  CFU·g<sup>-1</sup> d.m. substrate) and it was not significantly higher (Tukey's test;  $p > 0.05$ ) from the lowest average value recorded in the substrate with

the addition of 20 cm<sup>3</sup> Si + Ca under cultivation of the SA (A+) specimen ( $7.50 \times 10^5$  CFU·g<sup>-1</sup> d.m. substrate) – Figure 2.

The analysis of the results showed that in the strawberry gutter system, the addition of Si + Ca to the growing substrate did not significantly affect the number of actinobacteria (Tukey's test,  $p > 0.05$ ). It should be emphasized that actinobacteria is a group of bacteria frequently present in alkaline soil, with a large amount of organic matter. In addition, these bacteria are able to form spores, characterized by high resistance to stress and extraordinary metabolic activity. Actinobacteria isolated from soil also have the potential to inhibit the growth of some plant pathogens [Jeffrey 2008]. The next tested microorganisms in the experiment were *Azotobacter* bacteria. Due to the relatively high environmental requirements, similar to the requirements of arable crops, *Azotobacter* is referred to as the soil fertility index sometimes, and its presence in the environment can be a sign of good soil culture and biological activity, being also a good indicator for characteristics and evaluation of the soil. In ad-



**Fig. 2.** The effect of applied Si + Ca additives on the average number of actinobacteria in the cultivation substrate from different strawberry specimens cultivation



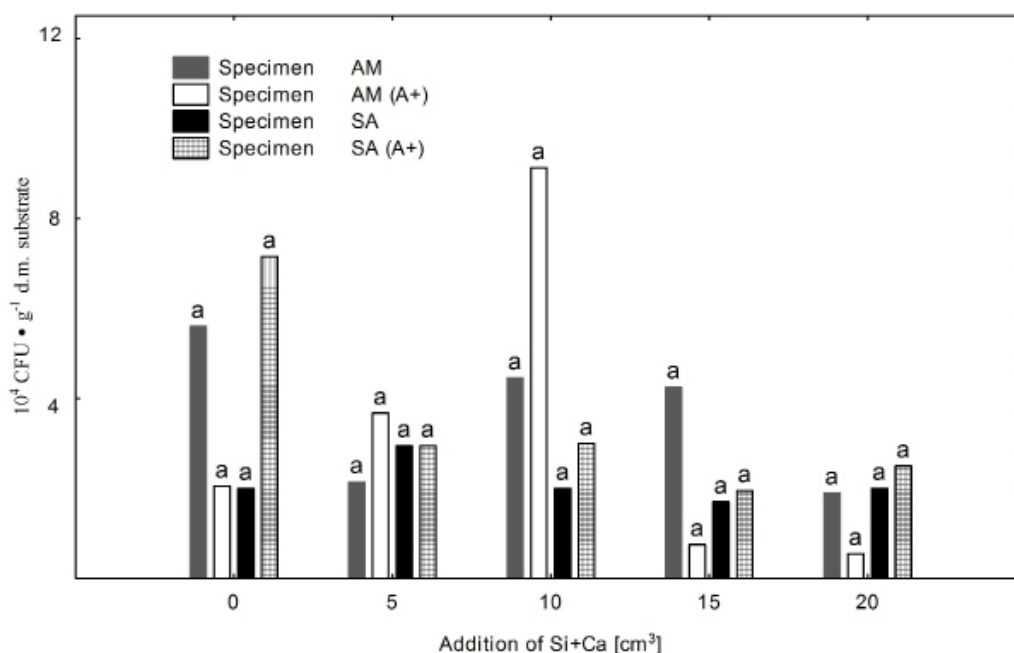
**Fig. 3.** The effect of applied Si + Ca additives on the average number of *Azotobacter* bacteria in the cultivation substrate from different strawberry specimens cultivation

dition, *Azotobacter* [Oelze 2000] enriches the soil with nitrogen (up to 20 kg nitrogen per ha per year), and synthesizing growth stimulants (vitamins, gibberellins, auxins) has a very beneficial effect on the growth and yielding of plants [Aquilanti et al. 2004]. A comparison of the results of 2-factor analysis of variance (ANOVA) describing the effect of various amounts of Si + Ca supplement and strawberries specimens on the number of *Azotobacter* bacteria indicates that both factors did not significantly affect the occurrence of *Azotobacter* (Tukey's test,  $p > 0.05$ ). This may prove that the cultivated substrate used in the guttering system of strawberries was characterized by a favorable reaction, as a result of the addition of Ca. The mentioned bacteria are very sensitive to acidification of the soil environment [Martyniuk et al. 2007]. Due to the relatively high environmental requirements, similar to the requirements of arable crops, *Azotobacter* is referred to as the soil fertility index sometimes, and its presence in the environment can be a sign of good soil culture and biological activity, being also a good indicator for characteristics and evaluation of the soil.

The highest average number of *Azotobacter* bacteria ( $2.60 \times 10^3$  CFU · g<sup>-1</sup> d.m. substrate) in the tested substrate was observed in the experimental variant where 10 cm<sup>3</sup> of Si + Ca was added to the substrate under cultivation of strawberries of the SA (A+) specimen. This value was not significantly higher (Tukey's test,  $p > 0.05$ ) from the lowest average, recorded in the substrate under cultivation of SA strawberry specimen with the addition of 5 cm<sup>3</sup> of Si + Ca ( $0.35 \times 10^3$  CFU · g<sup>-1</sup> d.m. substrate) – Figure 3.

In case of fungi, the analysis showed that the highest average number of fungi was found in the substrate with the addition of 10 cm<sup>3</sup> Si + Ca, under the AM (A+) specimen ( $9.12 \times 10^5$  CFU · g<sup>-1</sup> d.m. substrate) and the lowest in the substrate with 20 cm<sup>3</sup> Si + Ca under the AM (A+) strawberry specimen ( $3.2 \times 10^5$  CFU · g<sup>-1</sup> d.m. substrate). Due to the type of specimen of strawberries, the number of fungi in the cultivation substrate also did not differ significantly (Tukey's test,  $p > 0.05$ ) – Figure 4.

According to Myśków [1987], weaker growth of fungi in soil may be caused by competition with other



**Fig. 4.** The effect of applied Si + Ca additives on the average number of fungi in the cultivation substrate from different strawberry specimens cultivation

**Table 2.** The effect of Si + Ca and pH on the studied groups of microorganisms (Pearson's correlation)

Microorganisms	Si + Ca	pH
Total number of bacteria	$r = 0.60, p < 0.05$	$r = 0.07, p > 0.05$
Actinobacteria	$r = 0.32, p < 0.05$	$r = 0.09, p > 0.05$
<i>Azotobacter</i>	$r = 0.19, p > 0.05$	$r = 0.54, p < 0.05$
Fungi	$r = 0.12, p > 0.05$	$r = 0.36, p < 0.05$

microorganisms for food and higher soil pH. According to Wainwright [2003], silicon compounds can stimulate the growth of fungi in soil. However, due to the phytopathogenic and toxinogenic properties of fungi [Wielgosz and Szember 2006], their increased growth is an unfavorable phenomenon from the point of view of fertility of soils. It can be concluded that the analysis of the obtained data shows that the applied doses of 5, 10, 15 and 20 cm<sup>3</sup> of Si + Ca to the cultivation substrate used in the cultivation of various strawberry specimens did not significantly affect the number of studied groups of microorganisms, in comparison to the control samples (Tukey's test,  $p > 0.05$ ).

The results showing the effect of pH and Si + Ca in the growing substrate on the studied groups of microorganisms were evaluated using the  $r$  coefficient of the Pearson's correlation (Tab. 2).

Correlation analysis showed that the content of Si in the substrate had a significant effect on total number of bacteria ( $r = 0.60, p < 0.05$ ), actinobacteria ( $r = 0.32, p < 0.05$ ), whereas pH had a significant effect only on the number of fungi ( $r = 0.36, p < 0.05$ ) and *Azotobacter* bacteria ( $r = 0.54, p < 0.05$ ). These results confirm the already known fact that the presence of fungi and *Azotobacter* bacteria is dependent on the pH of the substrate. Lower pH of the substrate

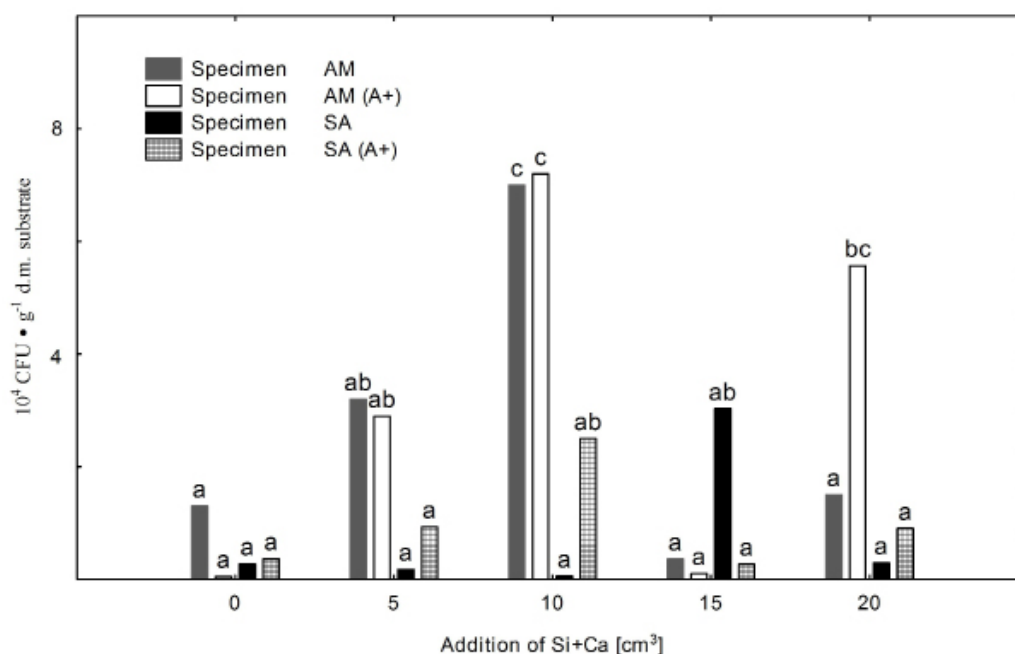


increases the growth of fungi and inhibits the growth of *Azotobacter* bacteria [Martyniuk et al. 2007, Natywa et al. 2014]. The mentioned bacteria [Limmer and Drake 1995] are very sensitive to acidification of the cultivation substrate environment. However, in the work of Karunakaran et al. [2013] there was found that nanosilica has a beneficial effect on the bacterial population and the nutritional value of the soil. Comparison of the number of analyzed microorganisms present in the effluents from gutters with cultivation substrate with strawberries, in individual experimental variants, indicates statistically significant differences only in the case of total number of bacteria and *Azotobacter* bacteria (Tukey's test,  $p < 0.05$ ). The analysis showed that the number of bacteria in 1 cm<sup>3</sup> of effluent ranged from  $0.19 \times 10^3$  to  $7.20 \times 10^5$  CFU·cm<sup>-3</sup>, for actinobacteria from  $0.04 \times 10^2$  up to  $0.90 \times 10^2$  CFU·cm<sup>-3</sup>, for *Azotobacter* bacteria from  $0.04 \times 10^2$  up to  $0.45 \times 10^2$  CFU·cm<sup>-3</sup>, and in the case of fungi from  $0.11 \times 10^2$  up to  $10.40 \times 10^2$  CFU·cm<sup>-3</sup>. In the case of bacteria, statistically significant domination was observed in the effluent from gutter with AM (A) strawberry specimen with the addition of 10 cm<sup>3</sup> Si + Ca (Tukey's test,

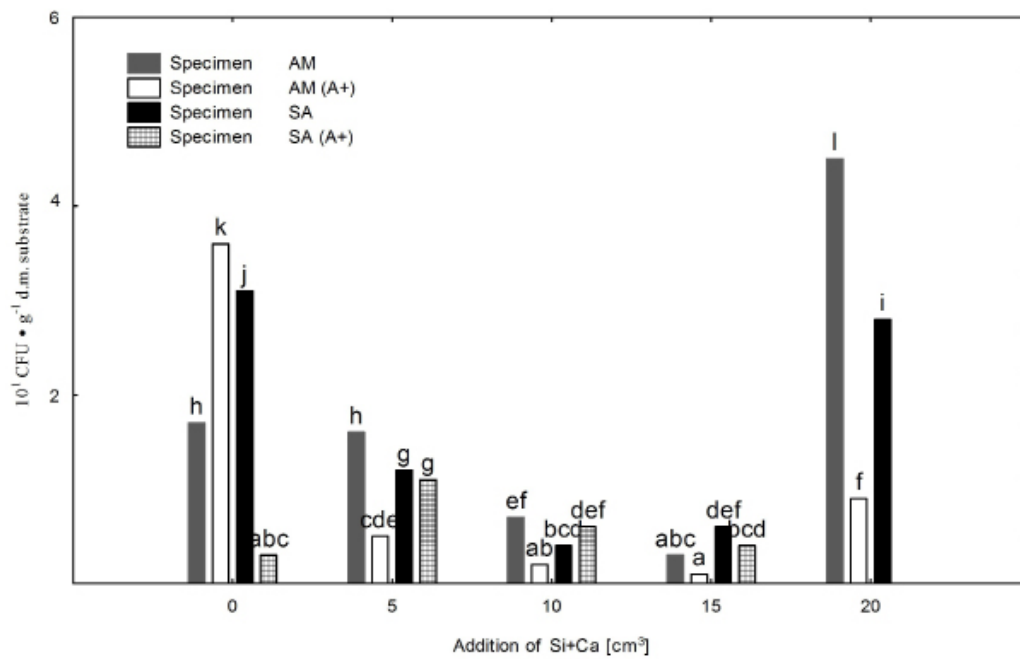
$p < 0.05$ ), relative to all other strawberry specimens: SA (A+), SA and 0, 5, 15 and 20 cm<sup>3</sup> Si + Ca additions applied to the substrate. The highest average of total number of bacteria recorded in effluent from AM (A+) strawberry specimen supplemented with 10 cm<sup>3</sup> of Si + Ca ( $72.00 \times 10^3$  CFU·cm<sup>-3</sup>) was statistically higher than the lowest average of total number of bacteria recorded in the control sample of the AM (A+) specimen cultivation ( $0.49 \times 10^3$  CFU·cm<sup>-3</sup>); Tukey's test,  $p < 0.05$  (Fig. 5).

The results of the ANOVA analysis show that significant differences in numbers of microorganisms were also observed in the effluents for *Azotobacter* bacteria and they resulted from the difference in their number between the effluent from the experimental variant with the addition of 15 and 20 cm<sup>3</sup> of Si + Ca to the substrate with SA (A+) and AM (A+) specimens and effluent from the experimental variant and effluent from the experimental variant with the addition of 20 cm<sup>3</sup> Si + Ca to the substrate with AM (A+) specimen (Tukey's test,  $p < 0.05$ ) – Figure 6.

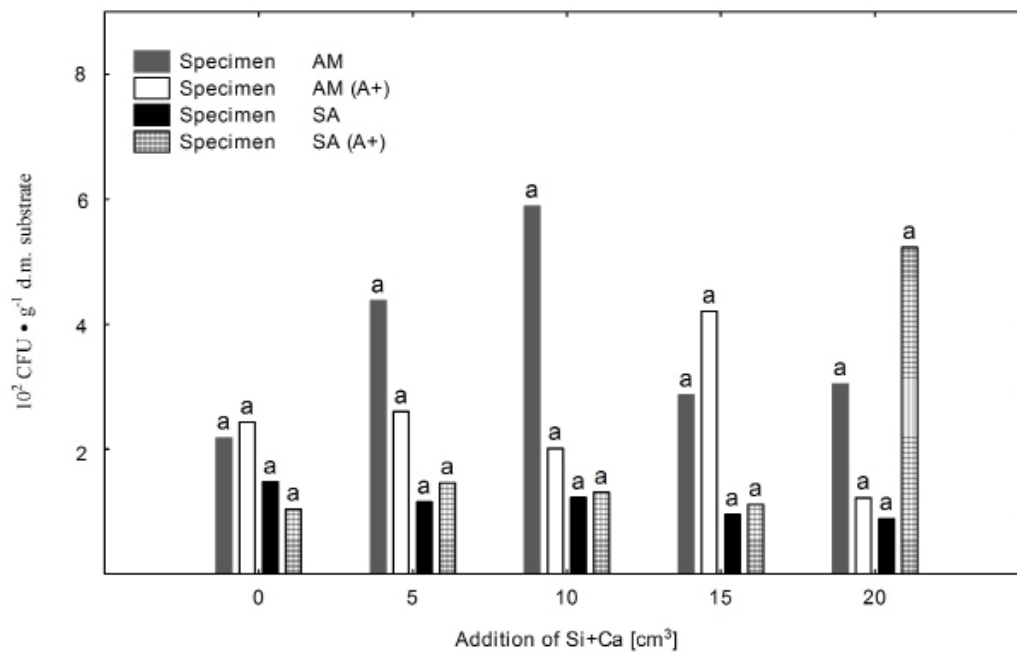
However, the analysis showed that the differences in the average number of fungi and actinobacteria



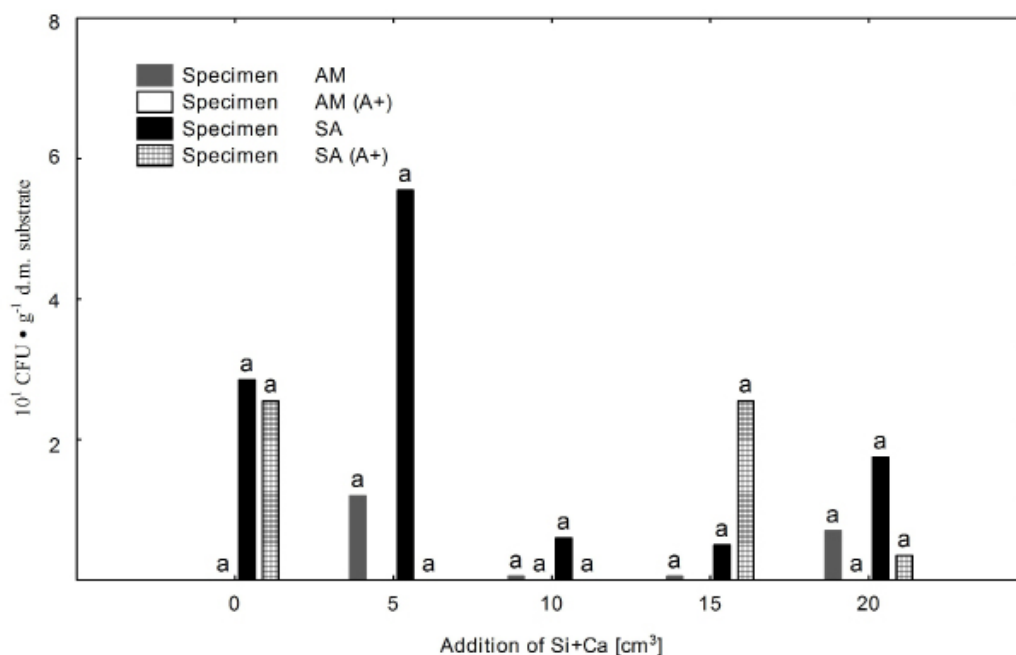
**Fig. 5.** The influence of Si + Ca additives applied to the cultivation substrate on the average of total number of bacteria in the effluent from gutters with various strawberry specimens cultivation



**Fig. 6.** Influence of Si + Ca additions applied to the cultivation substrate on the average number of *Azotobacter* bacteria in the effluent from gutters with various strawberry specimens cultivation



**Fig. 7.** The influence of Si + Ca additives applied to the cultivation substrate on the average number of fungi in the effluent from gutters with various strawberry specimens cultivation



**Fig. 8.** The influence of Si + Ca additives applied to the cultivation substrate on the average number of actinobacteria in the effluent from gutters with various strawberry specimens cultivation

in the examined effluents between the experimental variants were not statistically significant (Tukey's test,  $p > 0.05$ ). In case of fungi, their highest average number was recorded in the effluent from variant in which 10 cm<sup>3</sup> of Si + Ca were applied to the substrate with the AM strawberry specimen ( $0.58 \times 10^3$  CFU·cm<sup>-3</sup>) – Figure 7. In the case of actinobacteria, their highest average number was noted for the variant in which an addition of 5 cm<sup>3</sup> of Si + Ca was applied to the substrate with cultivation of the SA specimen ( $0.05 \times 10^3$  CFU·cm<sup>-3</sup>) – Figure 8. The results of the ANOVA analysis of the variance allowed to determine statistically significant differences (Tukey's test,  $p < 0.05$ ) between the average number of studied groups of microorganisms in the cultivation substrate with strawberry specimens in relation to their number in effluents. The biological significance of these observations requires further research, however, the present research already shows that the addition of silicon and calcium has a positive effect on the quantitative growth of the bacterial biota in the cultivation substrate used in soilless cultivation.

## CONCLUSIONS

The level of various additives in cultivation substrate affects plant growth and health, and the presence of microorganisms. High activity of microorganisms indicates good quality of the cultivation substrate and proper functioning of the processes carried out by soil microorganisms, which release nutrients available for crop plants. In the study, we assessed the effect of silicon and calcium addition to the cultivation substrate in soilless strawberry cultivation on the quantitative presence of a population of various groups of microorganisms in the substrate. Microbiological indicators used in the assessment of the substrate help to determine its productivity. In our work, we showed that the number of microorganisms associated with the cultivation substrate may be influenced by the addition of silicon and calcium. On the basis of the conducted tests, it was found that the studied cultivation substrate enriched with the Si + Ca additives is settled by soil microorganisms. The evaluation of the effect of silicon and calcium added to the substrate on the observed number

of the studied groups of microorganisms showed significant influence on the total number of bacteria in the substrate. Correlation analysis showed that the each addition of silicon to the substrate affects increase in the total number of bacteria in the substrate. The obtained results confirm that the cultivation substrate can be modified in order to increase the bacteria population, but this issue requires further research.

#### CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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