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RESEARCH PAPER

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THE EFFECT OF BIOCHAR AND CONSORTIA OF BENEFICIAL BACTERIA ON THE OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN CULTIVATION OF CUCUMBER, STRAWBERRY AND APPLE PLANTS

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ABSTRACT

The aim of the study was to evaluate the effect of newly developed microbial consortia on the degree of colonization of cucumber, strawberry, and apple roots by arbuscular mycorrhizal fungi and to determine the number of spores of these fungi in the rhizosphere soil. The following strains of beneficial bacteria were used in the 3 consortia: 1 – *Bacillus licheniformis* TES10B3, TES5B21, GOS10B9; *Streptomyces* sp. GOS5B1, 2 – *Pseudomonas* sp. Pi22B, Pi25C, *Klebsiella* sp. NAzot2, 3 – *Priestia* sp. TES5B10C, GOS5B22; *Bacillus licheniformis* GOS10B151. All consortia have been enriched with biochar. It was found that the use of Consortium 2 with biochar had asignificant effect on increasing the colonization of roots by arbuscular mycorrhizal fungi. Consortium 3 significantly increased the formation of mycorrhizal fungi spores in the rhizosphere soil in 2023, while in the following year the highest spore count was observed in the soil after the application of Consortium 2 with biochar. This beneficial effect makes both biochar and bacterial consortia recommended in the cultivation of horticultural plants to improve their growth and development and to improve the quality of soils, especially those that are poor in organic matter. Consortium 2 is recommended as a biostimulant for growing fruit and vegetable plants.

Keywords: root colonization, rhizosphere soil, spore count, biostimulant

INTRODUCTION

Feeding the ever-growing human population is one of the most significant challenges facing global agriculture. Environmental factors that limit food security and affect crop production are undoubtedly soil degradation [Lal 2020]. The decrease in the level of organic matter and nutrients in the soil has led producers to excessively use mineral fertilizers in order to maintain high-quality crops [Sas-Paszt et al. 2023]. However, this practice causes environmental pollution and greenhouse gas emissions, as well as a reduction in the population of beneficial microorganisms in the soil [Derkowska et al. 2024].

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Considering the above problems, biochar is becoming more widely used as a sustainable alternative in agriculture to improve soil quality and protect the natural environment [Jatuwong et al. 2024]. Used as a soil improver or as a component of biofertilizer to increase soil fertility and enhance its bio-physico-chemical properties, it can reduce the need for mineral fertilizers [Nepal et al. 2023, Kabir et al. 2023]. The components contained in biochar such as nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K) and high organic carbon content are essential for plant growth, and high porosity, low density, high capacity and neutral to alkaline pH make it increasingly used in the cultivation of horticultural and agricultural plants [Kabir et al. 2023]. The addition of biochar to soil, whether in the form of biofertilizers or composts, can increase soil organic carbon content and porosity, stabilize pH, immobilize toxic heavy metal elements, and increase soil microbial activity, which affects the development of the root system of crops and improves soil nutrient uptake [Nepal et al. 2023, Kabir et al. 2023, Sifton et al. 2023]. Biochar also plays an important role in reducing global greenhouse gas emissions and reducing atmospheric CO_2 concentration [Jabborova et al. 2021]. As a carrier of organic matter, it has been extensively studied for its effects on plants, however the effects of biochar on microbiome structure and biodiversity in different soil conditions have not yet been fully understood [Xu et al. 2023a]. Assessing the impact of biochar on soil microbial diversity in horticultural crops is crucial for maintaining soil health, enhancing productivity, and mitigating the adverse effects of intensive crop cultivation and climate change [Xu et al. 2023b]. When assessing soil quality, we often pay attention to the roles of microorganisms in the soil environment, the size and structure of their populations, and species diversity [Ren et al. 2022]. Different plant species grown in the same soil can affect the development of different groups of microorganisms [Zhang et al. 2022].

Soil microorganisms, through the decomposition of organic matter in the soil, make nutrients available to plants and are also responsible for the mineralization, adsorption and binding of elements supplied to the soil with both mineral and organic fertilizers [Li et al. 2022, Fuke et al. 2021]. The microbiome, occurring in the rhizosphere soil, i.e. the one directly surrounding plant roots, contains characteristic species of microorganisms, attracted by root exudates released into the soil [Perreault and Laforest-Lapointe 2022]. Sometimes it has been called the second plant genome [Zhang et al. 2022]. Direct interactions between crop roots and soil microorganisms have a significant impact on the growth, health and adaptation of plants to unfavorable environmental conditions [Petipas et al. 2021]. Plant growth-promoting bacteria (PGPB) are an important group of microorganisms that primarily colonize the rhizosphere of plants. They can support plant growth and development by increasing the bioavailability and uptake of carbon, nitrogen, and essential minerals from the soil [Racioppo et al. 2023]. They also contribute to increased plant yield, reduced pathogen infestation, and alleviate biotic and abiotic stress [Sharma et al. 2025]. In the natural environment, arbuscular mycorrhizal fungi (AMF) participate in mutualistic symbiotic associations with various crop species, are an essential component of the rhizosphere microbiome and play an important role in nutrient uptake from the rhizosphere and improve plant nutrition [Fall et al. 2022]. The use of mycorrhizal fungi as components of biostimulants and biofertilizers is a significant and environmentally friendly approach to modern agriculture [Shi et al. 2022, Sun et al. 2023]. AMF are widely used in ecological and sustainable crop production because they provide a number of benefits, e.g. they affect plant nutrition by providing micro and macro elements, participate in nitrogen fixation and participate in soil carbon cycling through the decomposition of organic matter [Ebbisa 2022]. Mycelial hyphae can affect soil structure and prevent its erosion, participate in the remediation of soils contaminated with heavy metals, support biodiversity and regulate water uptake by plants [Ebbisa 2022]. Studies by many authors have shown that the species composition and abundance of mycorrhizal fungi are regulated by the plants with which these fungi live in symbiosis, but the presence of AMF can also affect the abundance and composition of other species of microorganisms found in the rhizosphere soil [Emmett et al. 2021, Wang et al. 2021]. However, the conditions and mechanisms responsible for this relationship between mycorrhizal fungi and other microorganisms remain unknown. Ultimately, the effectiveness of nutrient uptake from the rhizosphere soil depends on this cooperation, which in turn affects the growth and development of plants and the biodiversity of the soil environment.

In this work, we undertook research on the influence of specific soil bacterial consortia and biochar on the level of colonization of cucumber, strawberry and apple plant roots by arbuscular mycorrhizal fungi naturally occurring in soil and the number of spores in the rhizosphere soil under the influence of their use.

MATERIALS AND METHODS

The research included 3 series of field experiments carried out at the Experimental field of the National Institute of Horticultural Research (INHORT) in Skierniewice, Poland (cucumber, strawberry) and the Experimental Orchard of INHORT in Dąbrowice near Skierniewice (apple).

Characteristics of the applied materials

Biochar was produced by rapid pyrolysis (at 280°C for 5 minutes) from coniferous wood chips containing 80% of organic matter and 20% of organic carbon.

Bacterial strains from SYMBIO BANK by INHORT, which have a beneficial effect on plant growth and development, were used to create consortia of microorganisms [Derkowska et al. 2023]: Consortium 1 – strains: *Bacillus licheniformis* TES10B3, TES5B21, GOS10B9; *Streptomyces* sp. GOS5B1; Consortium 2 – strains: *Pseudomonas* sp. Pi22B, Pi25C, *Klebsiella* sp. NAzot2; Consortium 3 – strains: *Priestia* sp. TES5B10C, GOS5B22, *Bacillus licheniformis* GOS10B151.

The consortia were applied in the form of a liquid suspension with a concentration of microorganisms of 10^6 cfu/ml after 72 hours of cultivation, with the exception of the *Streptomyces* sp. GOS5B1 strain after 7 days of cultivation.

Description of experiments

Cucumber, strawberry and apple plants were planted in podzolic soil with pH 6.2, and a quality rating of 3B, which contained macro-elements at the level of: P – 7.5, K – 12.4, Mg – 5.8 mg/100 g and microelements at the level of: B – 2.4, Cu – 4.8, Fe – 862, Mn – 75.5, Na – 4.35, Zn – 3.7 mg/1000 g, and the humus content was 1.2%. The preceding crop was mustard plants, and the soil was cultivated before planting the plants in accordance with the recommendations for cucumber, strawberry and apple plantations. The experiment was established in a randomized block design in four replications for each plant species. Prior to setting up the experiment granulated poultry manure at a rate of 200 g per plot for cucumber and strawberry, and 300 g per tree for apple, was applied. Biochar was applied at a rate of 2L per plot, mixed with the topsoil layer. Microbial consortia were applied in the root system zone around the growing plants by soil watering, at the following doses: cucumber and strawberry – 40 ml per plant, apple – 400 ml per tree The application of microbial consortia, biochar and manure was carried out in the spring of each year of the experiment, at the beginning of the growing season.

Experiment I. Cucumber seeds Octopus F1 cv. (Syngenta, Netherlands) were sown into the soil in May 2023 and 2024, respectively. Each plot ($2m^2$) constituted an experimental unit, where 14 seeds were sown with a spacing of 0.3 m within the row and 1 m between rows. Standard care treatments were performed during the experiment.

Experiment II. Frigo A+ strawberry plants of the Lycia variety (produced by Vivai Mazzoni Societa Agricola, Italy) were planted in April 2022 at a spacing of 0.9 m × 0.2 m. Each experimental combination consisted of 15 plants per plot. Standard maintenance procedures were performed on the strawberry experimental plots before planting, and in each of the two years of the experiment.

Experiment III. One-year-old maiden apple trees of the Gold Milenium variety, grafted onto M. 9 rootstock (produced by the Center for Elite Nursery Material in Prusy, Poland), were planted in May 2018 at a spacing of 2 m × 4 m. Until the experiment was established, standard maintenance procedures for commercial orchards were performed. The study involved 4-year-old apple trees with an experimental plot area of 12 m^2 (3 trees per plot) and standard maintenance procedures were performed on them.

The experiment consisted of 8 treatments, namely: Control (only granulated poultry manure at the beginning of the growing season), Biochar, Consortium 1, Consortium 2, Consortium 3, Consortium 1 + Biochar, Consortium 2 + Biochar, and Consortium 3 + Biochar.

Determination of root colonization by arbuscular mycorrhizal fungi naturally occurring in soil

The roots of apple, strawberry and cucumber plants (10 g from each replication), collected in July 2023 and 2024, were stained according to the method developed in the Department of Microbiology and Rhizosphere of INHORT [Derkowska et al. 2015]. Next, microscopic specimens were prepared and examined with a Nikon Eclipse 50i microscope (objectives with magnifications of 20×, 40×, 60×, 100×) and photographic records of the observed mycorrhizal structures were produced. The assessment of the degree colonization of the roots by arbuscular mycorrhizal fungi was performed by the method of Trouvelot et al. [1986]. Based on the results, the frequency of mycorrhiza occurrence (F%) was calculated using the computer program MYCOCALC, available on the website: http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE (Tables 1–6).

Counting of the number of spores of mycorrhizal fungi naturally occurring in rhizosphere soil

Samples of rhizosphere soil collected from the plants of every experimental combination, collected in July 2023 and 2024, were used to weigh out 100 g portions for further analyses. These were then placed in bottle containers and made up to 1 litre with distilled water. The resulting suspensions were shaken for approx. 1 hour

and placed in a refrigerator for 24 h at 4°C. After that, the soil solutions were filtered through a column of sieves (0.5 mm, 0.125 mm, 0.0063 mm, and 0.0045 mm). The fractions of soil remaining on the successive sieves were washed away with distilled water into Petri dishes (120 mm), to which sucrose (5 g per dish) was added. The samples thus prepared were examined using a Nikon SMZ 800 stereoscopic microscope, fishing out and counting spores of mycorrhizal fungi found in them [Błaszkowski 2008, Tables 1–6].

Statistical analysis

The results were statistically analysed by one-way analysis of variance in a random block design. Multiple comparisons of means for the combinations were performed with Tukey's test at a significance level of α = 0.05 using STATISTICA v.13.1 software (StatSoft Inc., 2011). Data not significantly different from each other are marked with the same letter.

RESULTS

Compared to the roots of control plants, the use of consortia of beneficial bacteria had a positive effect on the intensity and degree of root colonization by arbuscular mycorrhizal fungi present in the soil and the formation of their spores in the rhizosphere of cucumber, strawberry and apple plants.

Year 2023. Roots of cucumber plants after the application of Consortium 2 on its own, as well as in combination with biochar, were colonized to the greatest extent and most intensively by arbuscular mycorrhizal fungi. Similarly, applications of biochar and Consortium 1, as well as those applied in combination with biochar , also caused a significant increase in the colonization of roots by mycorrhizal fungi. Analyses of rhizosphere soil collected from experimental plots of cucumber plants showed that the combined application of Consortium 3 and biochar significantly increased the number of spores of arbuscular mycorrhizal fungi in the soil. On the other hand, the application of biochar and Consortium 2 had no significant effect on the number of spores in the rhizosphere soil of cucumber (Table 1).

The roots of strawberry plants after the combined application of Consortium 2 in combination with biochar were colonized to the greatest extent and most intensively by arbuscular mycorrhizal fungi. Similar results were obtained after the application of Consortium 2 on its own, while the application of biochar alone did not significantly affect the colonization of roots by AMF. Analysis of strawberry soil showed that the combined application of Consortium 3 and biochar significantly increased the number of spores of arbuscular mycorrhizal fungi in the soil. It is worth noting that a slightly lower number of these spores was found after the application of Consortium 2 in combination with biochar (Table 2).

The roots of apple trees after the application of Consortium 2 in combination with biochar and also after the application of Consortium 1 and 2 on their own were colonized to the greatest extent by arbuscular mycorrhizal fungi. In the roots of control trees, however, the colonization of roots was the lowest. Microscopic observations also allowed to state that the roots of apple trees were most intensively colonized by AMF after the application of Consortium 2 alone and in combination with biochar. Similarly to the soil from under cucumber and strawberry plants, also the soil from under apple trees after the combined application of Consortium 3 and biochar was characterized by the highest number of spores, and after the application of Consortium 2 and biochar alone, the analyzed soil contained the lowest number of spores (Table 3).

Year 2024. The combined use of Consortium 2 and biochar, and Consortium 2 applied alone significantly increased the colonization of cucumber roots by AMF. Only the roots of control plants were characterized by the lowest degree of root colonization by AMF. The highest relative intensity of mycorrhization was observed in the roots of cucumber plants after the application of Consortium 2, and Consortium 2 with biochar. Also the use of Consortium 2 with biochar significantly increased the number of spores of AMF in the rhizosphere soil of cucumber. Only in the control soil the number of spores was the lowest (Table 4).

In strawberry roots, it was found that the highest degree of root colonization was characteristic of plants after the combined application of Consortium 2 and biochar. The single application of Consortium 2 also significantly influenced the increase in root colonization by AMF, while the roots of control plants were colonized to the least extent. The combined application of Consortium 2 and biochar significantly influenced the intensity of colonization. Comparing the rhizosphere soil of strawberry, it was observed that the combined application of Consortium 2 and biochar increased the number of spores in the soil. Only the rhizosphere soil of control plants was characterized by the lowest number of spores (Table 5).

Table 1. Degree of cucumber root colonization by arbuscular mycorrhizal fungi and the number of spores in the rhizosphere soil after the application of beneficial bacteria and biochar in 2023 (INHORT Experimental Field in Skierniewice, 2023)

Treatment	F (%)	M (%)	m (%)	Spore count (per 100 g soil)
Control	15.56 a	1.02 a	5.90 a	9 a
Biochar	33.33 b-d	2.39 ab	7.10 a	14 a
Consortium 1	33.33 b-d	2.35 ab	6.97 a	32 b
Consortium 2	41.11 d	2.79 b	6.94 a	11 a
Consortium 3	22.22 ab	1.73 ab	7.89 a	28 b
Consortium 1 + Biochar	36.67 cd	2.75 b	7.51 a	29 b
Consortium 2 + Biochar	45.55 d	3.20 b	6.92 a	36 b
Consortium 3 + Biochar	25.56 a-c	2.18 ab	8.60 a	51 c

F – mycorrhizal frequency, M – relative mycorrhizal frequency (for the whole sample), m – absolute mycorrhizal frequency (for the segments in which there was some evidence of colonization by mycorrhizal fungi)

Table 2. Degree of strawberry root colonization by arbuscular mycorrhizal fungi and the number of spores in the rhizosphere soil after the application of beneficial bacteria and biochar in 2023 (INHORT Experimental Field in Skierniewice, 2023)

Treatment	F (%)	M (%)	m (%)	Spore count (per 100 g soil)
Control	12.22 a	0.90 a	6.94 a	12 a
Biochar	20.0 a	1.71 ab	8.56 a	22 b
Consortium 1	46.67 c	2.52 bc	5.36 a	42 cd
Consortium 2	51.11 cd	3.62 c	7.08 a	29 b
Consortium 3	34.44 b	1.53 ab	4.45 a	39 c
Consortium 1 + Biochar	35.56 b	2.69 bc	4.47 a	34 c
Consortium 2 + Biochar	58.89 d	3.70 c	6.26 a	51 d
Consortium 3 + Biochar	32.22 b	2.24 a-c	6.90 a	65 e

See note under table 1

Table 3. Degree of apple root colonization by arbuscular mycorrhizal fungi and the number of spores in the rhizosphere soil after the application of beneficial bacteria and biochar in 2023 (INHORT Experimental Orchard in Dąbrowice, 2023)

Treatment	F (%)	M (%)	m (%)	Spore count (per 100 g soil)
Control	12.22 a	0.99 a	8.02 a	24 a
Biochar	25.56 b	2.22 a-c	8.77 a	33 a
Consortium 1	42.22 c	2.89 bc	6.84 a	91 cd
Consortium 2	40.0 c	3.19 c	7.94 a	30 a
Consortium 3	27.78 b	1.88 a-c	6.81 a	65 b
Consortium 1 + Biochar	13.33 a	1.28 ab	8.78 a	74 bc
Consortium 2 + Biochar	40.0 c	2.78 bc	6.99 a	100 d
Consortium 3 + Biochar	27.78 b	2.20 a-c	7.90 a	128 e

See note under table 1

Table 4. Degree of cucumber root colonization by arbuscular mycorrhizal fungi and the number of spores in the rhizosphere soil after the application of beneficial bacteria and biochar in 2024 (INHORT Experimental Field in Skierniewice, 2024)

Treatment	F (%)	M (%)	m (%)	Spore count (per 100 g soil)
Control	25.56 a	2.18 a	8.60 b	13 a
Biochar	35.56 b	2.69 ab	4.47 a	24 ab
Consortium 1	46.67 c	3.21 ab	6.85 ab	34 bc
Consortium 2	58.89 d	3.70 b	6.26 ab	44 cd
Consortium 3	42.22 bc	3.08 ab	7.28 ab	38 c
Consortium 1 + Biochar	46.67 c	3.12 ab	6.67 ab	43 c
Consortium 2 + Biochar	58.89 d	3.24 ab	5.50 ab	56 d
Consortium 3 + Biochar	41.11 bc	2.70 ab	6.57 ab	36 c

See note under table 1

Table 5. Degree of strawberry root colonization by arbuscular mycorrhizal fungi and the number of spores in the rhizosphere soil after the application of beneficial bacteria and biochar in 2024 (INHORT Experimental Field in Skierniewice, 2024)

Treatment	F (%)	M (%)	m (%)	Spore count (per 100 g soil)
Control	22.22 a	1.78 a	7.96 b	14 a
Biochar	34.45 b	2.40 ab	6.88 ab	26 b
Consortium 1	50.0 cd	3.57 ab	6.12 ab	36 bc
Consortium 2	60.93 de	3.38 ab	5.70 ab	53 d
Consortium 3	44.44 bc	2.32 ab	5.21 a	44 cd
Consortium 1 + Biochar	44.45 bc	3.14 ab	7.06 ab	47 cd
Consortium 2 + Biochar	62.22 e	3.69 b	5.91 ab	66 e
Consortium 3 + Biochar	44.44 bc	2.50 ab	5.58 ab	38 c

See note under table 1

Table 6. Degree of apple root colonization by arbuscular mycorrhizal fungi and the number of spores in the rhizosphere soil after the application of beneficial bacteria and biochar in 2024 (INHORT Experimental Orchard in Dąbrowice, 2024)

Treatment	F (%)	M (%)	m (%)	Spore count (per 100 g soil)
Control	23.33 a	2.11 a	8.95 b	23 a
Biochar	33.33 ab	2.30 a	6.84 ab	34 a
Consortium 1	43.33 bc	2.81 ab	6.50 ab	35 a
Consortium 2	61.11 d	3.95 b	6.48 ab	67 bc
Consortium 3	46.67 c	3.12 ab	6.65 ab	56 b
Consortium 1 + Biochar	48.89 c	3.83 b	7.87 ab	81 c
Consortium 2 + Biochar	65.56 d	3.77 b	5.76 a	130 d
Consortium 3 + Biochar	48.89 c	3.14 ab	6.42 ab	64 b

See note under table 1

In the cultivation of apple trees, it was found that the combined application of Consortium 2 with biochar, and the application of Consortium 2 on its own significantly increased the colonization of roots by arbuscular mycorrhizal fungi. The application of Consortium 2 had the greatest effect on the intensity of mycorrhizal fungi colonization of apple tree roots. Soil analysis showed that under the influence of the combined application of Consortium 2 and biochar, the number of spores was the highest. However, the application of biochar and Consortium 1 did not significantly affect the number of spores in the rhizosphere soil of apple trees. The lowest number of spores was observed in the rhizosphere control soil (Table 6).

DISCUSSION

The results of our studies indicate the high effectiveness of using Consortium 2 and biochar in increasing the colonization of cucumber, strawberry and apple plant roots by arbuscular mycorrhizal fungi. Biochar applied together with Consortia 2 and 3 increased the formation of spores of these fungi in the rhizosphere soil of the studied plant species. These results are also confirmed by studies of other authors.

Due to the beneficial effect of biochar on the growth and yield of perennial plants, in recent years there has been an increased interest in its use in the field cultivation of horticultural plants, mainly fruit trees [Frąc et al. 2022]. Less is known, however, about the interaction of biochar with soil microorganisms and its impact on soil biodiversity, which is one of the factors influencing soil functioning and soil biodiversity [Chen et al. 2022].

Videgain-Marco et al. [2021] conducted a study to determine the effect of biochar derived from grapevine shoots on the activity of arbuscular mycorrhizal fungi and the effect of their multiplication in soil on plant resistance to water stress. The results showed an overall increase in AMF frequency and activity after the application of biochar produced at 400 °C in sandy-loam substrate. They also found that the addition of biochar increased AMF root colonization and spore count, and the developed inoculum containing arbuscular mycorrhizal fungi increased root colonization and spore count in the soil where lettuce plants were grown under limited irrigation conditions. Malik et al. [2019] assessed the effect of biochar application on the occurrence of arbuscular mycorrhizal fungi in the soil and roots of wheat and maize. The results showed that AMF root colonization in maize was significantly greater than in wheat, and biochar application significantly increased both the number of spores in the soil and the number of spores in the roots of wheat and maize plants, compared to the control plants and soil. The authors suggest that the use of biochar increased the number of native populations of arbuscular mycorrhizal fungi, occurring in wheat and maize crops growing in alkaline calcareous soil with low fertility, in both the soil and the roots of these plants. The results obtained in our study are consistent with those obtained by Videgain-Marco et al. [2021] and Malik et al. [2019].

Compared to the control plants, biochar application increased the degree of root colonization and the number of AMF spores in the rhizospheric soil of the tested cucumber, strawberry and apple plants. Studies by other authors have shown that the use of biochar increases soil microbial activity and improves its chemical and physical properties [Qayyum et al. 2020, El Nahhas et al. 2021]. Chen et al. [2025] studied the use of biochar to accelerate the rate of colonization of American ginseng (*Panax quinquefolius* L.) roots by arbuscular mycorrhizal fungi and to explore its potential mechanisms. The results of this study indicate that the use of biochar accelerates the colonization of AMF in roots. It was also found that biochar affected the presence of potentially beneficial microorganisms such as *Sphingobium*, *Sphingomonas*, and *Novosphingobium*, which accelerate the colonization of plant roots by arbuscular mycorrhizal fungi. This is confirmed by the results of our research, in which the use of beneficial microorganisms such as *Bacillus licheniformis*, *Pseudomonas* sp., *Klebsiella* sp., *Priestia* sp., which are components of Consortia 1 and 2, increased the degree of AMF colonization of cucumber, strawberry and apple roots and the number of spores in the rhizospheric soil.

Agricultural practice is moving towards the combined use of biochar and other organic and inorganic fertilizers [Bai et al. 2022] and beneficial soil microorganisms [Sas-Paszt et al. 2023]. The study conducted by Ning et al. [2019] aimed to determine the effect of mycorrhizal fungi and *Pseudomonas* sp. interactions on phosphorus (P) and nitrogen (N) uptake and on the root length, surface area and volume of celery plants after biochar application at low and high phosphorus fertilization. They observed that the rate of root colonization by AMF fungi increased as a result of the combined use of *Pseudomonas* sp. and biochar. Our results also indicate that the combined application of Consortium 2, which includes, among others, *Pseudomonas* sp. (Pi22B, Pi25C) bacteria and biochar had the greatest impact on the extent of root colonization by arbuscular mycorrhizal fungi of cucumber, strawberry and apple. In their study, Ashraf and Chen [2023] assessed the effect of biochar application on soil properties, the

growth of wild rye (*Elymus elymoides*) and diversity of mycorrhizal fungi. Soil samples were treated with different doses of biochar and the inoculated with arbuscular mycorrhizal fungi. The results confirmed their assumptions that the use of biochar had a significant effect on the growth and diversity of mycorrhizal fungi. The combined use of biochar and AMF significantly increased the number of mycorrhizal fungi such as: *Rhizophagus* to 62.67%, *Glomus* to 67.67%, *Clarediogous* to 38.33% and *Redochera* to 16.67%. Other results were obtained by Sun et al. [2022] who studied the effects of biochar and arbuscular mycorrhizal fungi on the growth of maize (*Zea mays* L.), mineral content and chlorophyll content of the plants. They observed that biochar application to soil significantly reduced mycorrhizal colonization by 40.58% in maize roots, accompanied by a significant decrease in mycorrhizal dependence from 80.57% to –28.67%. Zhao et al. [2024] evaluated the effects of biochar and arbuscular mycorrhizal fungi on the growth, physiological traits and genetic expression of rice plants subjected to Cd stress. The results showed that biochar significantly increased the rate of mycorrhizal colonization by 22.19%. Wen et al. [2024] conducted a study to determine the effects of biochar and the application of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* on prairie grass growth and soil quality. They found that the combined application of biochar and *Rhizophagus irregularis* significantly changed the microbiological composition of the rhizosphere soil and its quality. It also significantly increased the number of beneficial *Bacillus* sp. bacteria. In our experiments, a different mechanism was used, in which the included strains of *Bacillus licheniformis* bacteria (Consortium 1 and 2) significantly increased the colonization of cucumber, strawberry and apple plant roots and the number of AMF spores in the rhizosphere soil.

CONCLUSIONS

The use of beneficial bacterial consortia positively affected the degree and intensity of root colonization by arbuscular mycorrhizal fungi in cucumber, strawberry, and apple plants compared to control plants.

In both study years (2023–2024), the use of Consortium 2, alone or in combination with biochar, was most effective in increasing the degree of AMF colonization of the roots of the studied plant species.

In 2023, the highest number of AMF spores in the rhizosphere soil was observed after the combined application of Consortium 3 and biochar, while in 2024, the combined application of Consortium 2 and biochar significantly increased both root colonization and the number of AMF spores in all studied plant species.

The results indicate that the selection of appropriate microorganisms and their combination into consortia, e.g. *Pseudomonas* sp. Pi22B, Pi25C, *Klebsiella* sp. NAzot2a (Consortium 2), can effectively stimulate the development of AMF both in roots and in soil, and their effect can be enhanced by the use of biochar.

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CONFLICT OF INTEREST

The authors declare that they do not have any known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study concept and design. L.S.-P. was responsible for the experimental design, methodology, supervision, and administration of the project. E.D. and B.S. conducted the study material preparation and laboratory evaluation. K.G. and S.G. performed data collection and statistical analysis, and together with E.D., they developed the tables. The first draft of the manuscript was written by E.D., and all authors commented on subsequent versions of the manuscript. K.G. provided linguistic proofreading. All authors read and approved the final version of the manuscript.

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