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THE INFLUENCE OF BIOSTIMULANTS ON THE RHIZOSPHERIC MICROORGANISMS OF SCORZONERA (*Scorzonera hispanica* L.)

Elżbieta Patkowska

Department of Plant Protection, Leszczyńskiego 7, 20–069 Lublin, University of Life Sciences in Lublin, Poland

ABSTRACT

Scorzonera (*Scorzonera hispanica* L.) is a particularly valuable species among little-known and rarely cultivated vegetables. It is a root vegetable of high dietary and nutritional values. The suitable microbiological activity of the soil favors the growth and development of scorzonera. Biostimulants can positively affect the communities of rhizospheric microorganisms of cultivated plants, including this important vegetable. The studies established the influence of biostimulants on the microbial communities in the scorzonera rhizosphere. Before setting up the field experiment, scorzonera seeds were dressed with fungicide Zaprawa Nasienna T 75 DS/WS or biostimulants Beta-Chikol, Bio-Algeen S-90, and Asahi SL. The laboratory microbiological analyses of scorzonera rhizosphere soil were conducted and determined the total population of bacteria and fungi. The obtained rhizosphere isolates of fungi *Albifimbria*, *Clonostachys*, *Epicoccum*, *Penicillium*, and *Trichoderma* sp. were tested to check the influence on fungi pathogenic to scorzonera (*Fusarium culmorum, Fusarium oxysporum*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani*). The experiments showed that biostimulants, especially Asahi SL and Beta-Chikol, favored the development of rhizobacteria populations (including *Bacillus* sp. and *Pseudomonas* sp.). All biostimulants (Beta-Chikol, in particular) and the fungicide decreased the population of rhizospheric fungi and limited the occurrence of polyphagous fungi in the rhizosphere of scorzonera. Biostimulant Beta-Chikol and fungicide Zaprawa Nasienna T 75 DS/WS were most effective in stimulating the development of antagonistic fungi. *Clonostachys rosea*, *Trichoderma* sp., and *Albifimbria verrucaria* predominated as antagonistic rhizospheric fungi.

Key words: high-inulin root vegetable, Bio-Algeen S-90, Asahi SL, Beta-Chikol, rhizospheric microorganisms

INTRODUCTION

Scorzonera (*Scorzonera hispanica* L.), commonly called black salsify, whose juice was used in folk medicine as a remedy against viper poison, is an especially valuable species among little-known and rarely cultivated vegetables. It has high dietary and nutritious values, the taproot being edible. This plant belongs to the aster family (*Asteraceae*). Wildly growing forms of scorzonera occur in Spain, in the south of Germany and France, and in Caucasia [Konopiński 2003]. The species *Scorzonera purpurea* L., a protected plant in the Polish Red Data Book as an endangered species, can be found in Poland [Zarzycki and Szeląg 2006]. The cultivated species *S. hispanica* L. is divided into botanical varieties: *S. hispanica* var. *asphodelodes* Wallr., which has linear leaves, and *S. hispanica* var. *glastifolia* W., with lancet-shaped and elongated leaves [Konopiński 2003].

It owes its pro-health and medicinal properties to a high content of polyphenolic acids, vitamins, min-

elzbieta.patkowska@up.lublin.pl

eral elements, and inulin, the latter being a glycoside, a soluble dietary fiber element [Dolota and Dąbrowska 2004]. Inulin is a precious dietary element. This is why therapeutics use scorzonera and its products as a pro-health and light food for people with diabetes [Kaur and Gupta 2002, Roberfroid 2002]. Cultivating this vegetable should be to obtain high and good quality yields of roots [Konopiński 2003]. The quality and healthiness of root vegetables, including scorzonera, depends, for example, on soil-borne pathogens [Patkowska 2020, Patkowska et al. 2022]. The suitable microbiological activity of the soil promotes the growth and development of scorzonera.

Soil microorganisms interact with the plant and form a dynamic system in which, e.g., processes of direct pathogen growth limitation and processes exerting an indirect effect on the growth of both organisms co-occur. These processes enhance the induction of plant resistance to pathogens and regulate mutual metabolite production [Grant and Jones 2009, Sood et al. 2020]. Various soil microorganisms and natural compounds can elicit plant resistance to phytopathogens [Walters et al. 2007, Grant and Jones 2009, Jamiołkowska 2020]. The synthesis of specific PR proteins (pathogenesis-related proteins), phytoalexins, and the activity of defense enzymes is initiated in plant cells [Selitrennikoff 2001, Walters et al. 2007]. These enzymes inhibit the germination of fungal spores and degrade the cell walls of phytopathogens [Balasubramanian et al. 2012, Chandrasekaran et al. 2017]. Among soil microorganisms, an essential role in limiting the development of plant pathogens is played by antagonistic rhizobacteria, especially *Bacillus* sp. and *Pseudomonas* sp., as well as rhizospheric fungi, in particular by *Trichoderma* sp. and *Clonostachys* sp. [Ma et al. 2012, Abbo et al. 2014, Patkowska et al. 2020, Sood et al. 2020, Patkowska 2021].

Biostimulants can positively affect the communities of rhizospheric microorganisms of cultivated plants, including scorzonera [Drobek et al. 2019, Patkowska 2021]. These are different kinds of natural substances used in plant protection to facilitate their growth and development through increased tolerance to biotic and abiotic stress and more efficient uptake of nutrients [du Jardin 2015]. Biostimulants also include microorganisms that change the species composition of organisms occurring in the soil or plants [Drobek et al. 2019]. These include fungi [Colla et al. 2015, Mukherjee et al. 2013] and bacteria [Gaiero et al. 2013, Zhao et al. 2018]. Biostimulants can directly influence agrophages, including soil-borne bacteria and fungi. Such biostimulants as, for example, Bio-Algeen S-90, Beta-Chikol, Asahi SL, and Tytanit found their application in the cultivation of different species of vegetables, cereals, and grasses [Sosnowski et al. 2020, Malik et al. 2021, Patkowska et al. 2022].

Field and laboratory studies aimed to establish the influence of biostimulants Bio-Algeen S-90, Beta-Chikol, and Asahi SL's influence on the scorzonera rhizosphere's microbial communities. Moreover, the studies determined the population of antagonistic fungi that occurred in the rhizosphere of this plant and inhibited the growth of selected pathogenic fungi.

MATERIAL AND METHODS

The investigations were conducted in 2014–2016 in Poland (in the south-eastern part, Lublin region – 51°23' N, 22°56' E). The rhizosphere soil from under the cultivation of *S. hispanica* cv. Duplex was studied. The vegetable was grown on ridges with winter wheat as its forecrop. The field experiment was set up in 4 replications, and the experimental plots were 14 m^2 each. Scorzonera seeds were sown in the first 10-day period of May in the amount of 12 $kg \cdot ha^{-1}$ [Patkowska et al. 2022].

Before seed sowing, scorzonera seeds were dressed with biostimulants following the producers' recommendations: Bio-Algeen S-90 – 15 $ml \cdot kg^{-1}$ seeds, Beta-Chikol – 100 $ml \cdot kg^{-1}$ seeds, Asahi SL – 50 $ml \cdot kg^{-1}$ seeds. The experiment also used fungicide Zaprawa Nasienna T 75 DS/WS (a.s. – tiuram 75%) – 5 $g \cdot kg^{-1}$ seeds. Bio-Algeen S-90 contains microelements and macroelements, vitamins, alginic acids, and amino acids. It is an extract from *Ascophyllum nodosum*. The active substance of Beta-Chikol is chitosan, while Asahi SL contains 0.2% sodium ortho-nitrophenolate, 0.3% sodium para-nitrophenolate, and 0.1% sodium 5-nitroguaiacolate [Patkowska et al. 2022].

According to the method described by Patkowska [2021] for scorzonera and Patkowska et al. [2020] for carrot, the microbiological laboratory analysis of rhizosphere soil was performed. Ten weeks after the sowing of scorzonera, 40 plants (10 from each plot) were dug out from each experimental combination. The rhizospheric soil was the soil adhering to the roots of the plants. 10 g of the soil (4 replications for each experimental variant) was weighed for the microbiological analysis.

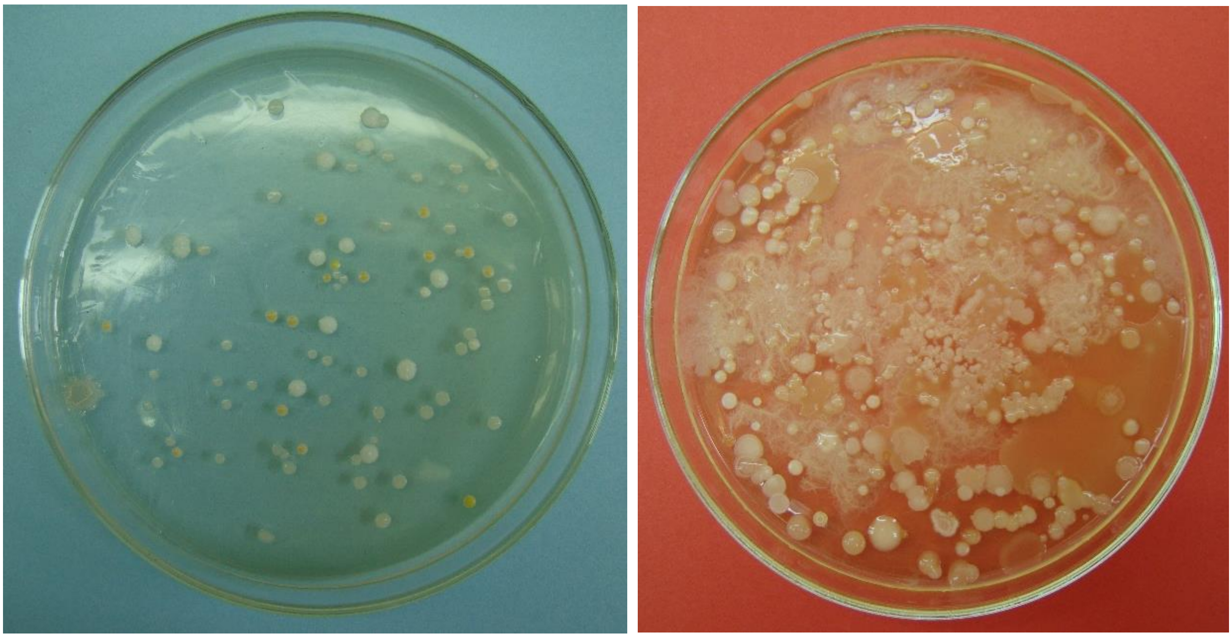
Then, the total population of bacteria in the Nutrient agar medium was established in Petri dishes in laboratory conditions following the method described by Patkowska et al. [2020] and Patkowska [2021]. The population of *Bacillus* sp. bacteria was determined using Tryptic soy agar, while *Pseudomonas* sp. was established on Pseudomonas F agar. Martin's medium was used to determine the population of fungi in the rhizosphere of fungi. After incubation (2–5 days at 20–22°C), the number of bacterial and fungal colonies was determined and converted into CFU/g soil DW (colony forming units/g soil dry weight) [Patkowska 2020]. Fungal cultures were transferred to sterile Petri dishes with PDA (potato dextrose agar) medium and incubated at 20–22°C for 2–3 weeks. In the next stage, the fungi were identified under a microscope by determining their morphological structures, such as conidia, conidiophores, and mycelium. The genus and species of fungi were marked based on the keys by Leslie and Summerell [2006], Ramirez [1982], and monographs provided by Patkowska et al. [2020]. The number and percentage of fungi species obtained were calculated.

The isolates of fungi such as *Trichoderma* sp., *Penicillium chermesinum*, *P. chrysogenum*, *P. lividum* *Clonostachys rosea*, *Albifimbria verrucaria*, *Epicoccum nigrum*, obtained as a result of the microbiological analysis of the rhizospheric soil, were tested concerning the fungi pathogenic towards scorzonera, such as *Fusarium culmorum, F. oxysporum*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. Those laboratory tests were conducted on a PDA medium according to the method described by Patkowska [2021], Patkowska et al. [2020], and Mańka and Mańka [1992]. Following the method described by Mańka and Kowalski [1968], the biotic effect of the fungi was determined after ten days of their growth. This analysis allowed to determine the number of antagonistic fungi isolated from the rhizosphere of scorzonera.

Results concerning the number of rhizosphere bacteria and fungi were statistically analyzed. The means were compared using the least significant differences based on Tukey's test ($p \leq 0.05$). Statistical calculations were done using Statistica, version 7.1 (StatSoft, Krakow, Poland).

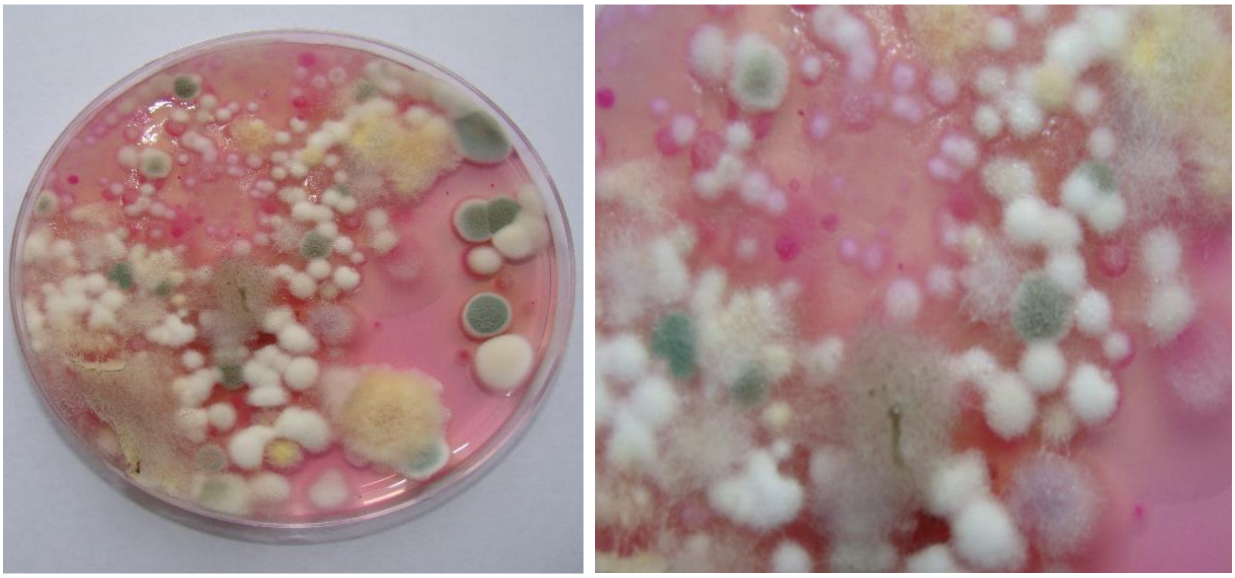
RESULTS AND DISCUSSION

The quantitative and qualitative composition of rhizospheric microorganisms obtained through the microbiological analysis of the soil (Fig. 1 and 2) varied and was related to the kinds of biostimulants and fungicides applied (Tab. 1). The applied preparations stimulated the development of bacteria, including *Bacillus* sp. and *Pseudomonas* sp., in the rhizosphere of scorzonera. Their population was statistically significantly more significant than in control. Depending on the variant of the experiment, the mean population of bacteria ranged from 3.25×10^6 to 9.67×10^6 CFU/g of soil DW (Tab. 1). The biggest total population of bacteria occurred in the rhizosphere after the application of Beta-Chikol and Asahi SL (on average, 9.67×10^6 and 8.45×10^6 CFU/g of soil DW, respectively). A slightly smaller population of bacteria was obtained after the application of Bio-Algeen S90 (on average, 7.59×10^6 CFU/g of soil DW) and Zaprawa Nasienna T 75 DS/WS (on average, 7.95×10^6 CFU/g of soil DW). The smallest population of bacteria was found in the rhizospheric soil taken from the control combination. A similar relation was observed for rhizobacteria *Bacillus* sp. and *Pseudomonas* sp. The population of these bacteria ranged, respectively, from 1.75×10^6 to 6.27×10^6 CFU/g of soil DW and from 0.77×10^6 to 3.28×10^6 CFU/g of soil DW, and it was statistically significantly more prominent than in control. Biostimulants Beta-Chikol and Asahi SL favored the development of *Bacillus* sp. and *Pseudomonas* sp. populations in the rhizosphere of scorzonera the most. Bio-Algeen S90 and Zaprawa Nasienna T 75 DS/WS stimulated the development of these bacteria to a slightly smaller degree. A reverse relation was observed for the population of rhizospheric fungi. After applying these preparations, the fungi population was statistically significantly smaller than in control, and it ranged, on average, from 1.23×10^3 to 8.52×10^3 CFU/g of soil DW. Beta-Chikol proved to be the most effective in limiting the development of the rhizospheric fungi population (1.23×10^3 CFU/g of soil DW). The other preparations were also effective in decreasing the



(a) (b)

Fig. 1. Bacterial colonies in Petri dishes isolated from the rhizosphere of scorzonera: (a) – *Pseudomonas* sp.; (b) – *Bacillus* sp.; (photo by E. Patkowska)



(a) (b)

Fig. 2. Fungi in Petri dishes isolated from the rhizosphere of scorzonera: – colonies of fungi; (b) – mycelium of rhizospheric fungi; (photo by E. Patkowska)

Table 1. Number of bacteria and fungi isolated from the rhizosphere of scorzonera

Experimental treatment	Total CFU of bacteria ($10^6 \cdot g^{-1}$ of soil DW)				CFU of *Bacillus* sp. ($10^6 \cdot g^{-1}$ of soil DW)				CFU of *Pseudomonas* sp. ($10^6 \cdot g^{-1}$ of soil DW)				Total CFU of fungi ($10^3 \cdot g^{-1}$ of soil DW)			
	2014	2015	2016	mean	2014	2015	2016	mean	2014	2015	2016	mean	2014	2015	2016	mean
Asahi SL	8.20b	8.32b	8.84b	8.45B	5.00b	5.14b	5.34b	5.16B	2.92a	3.16a	3.35a	3.13A	2.38b	3.15b	4.64b	3.39B
Beta-Chikol	9.46a	9.60a	9.96a	9.67A	6.15a	6.25a	6.42a	6.27A	3.20a	3.24a	3.40a	3.28A	1.24c	1.00c	1.45c	1.23C
Bio-Algeen S-90	5.94b	7.98b	8.84b	7.59B	3.86b	4.80b	4.90b	4.52B	1.80b	2.82b	3.06b	2.56B	3.92b	3.80b	4.92b	4.21B
Zaprawa Nasienna T 75 DS/WS	6.25b	8.45b	9.15b	7.95B	4.12b	5.02b	5.48b	4.87B	2.00b	3.14a	3.25a	2.80B	3.84b	3.90b	5.15b	4.30B
Control	2.35c	3.16c	4.25c	3.25C	1.15c	1.94c	2.15c	1.75C	0.52c	0.74c	1.04c	0.77C	7.76a	8.54a	9.26a	8.52A

Homogeneous groups according to Tukey's test ($p \leq 0.05$) for comparison of means within factors and their interactions.

Table 2. Fungi isolated from the rhizosphere of scorzonera

Fungus species	Experimental treatment / Fungi isolated from the rhizosphere of scorzonera (number of isolates)																				sum of isolates
	Asahi SL				Beta-Chikol				Bio-Algeen S-90				Zaprawa Nasienna T 75 DS/WS				control				
	2014	2015	2016	total	2014	2015	2016	total	2014	2015	2016	total	2014	2015	2016	total	2014	2015	2016	total	
Acremonium rutilum W. Gams	–	–	–	–	–	–	–	–	1	2	–	3	–	–	–	–	5	5	3	13	16
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	4	3	5	12	4	6	5	15	3	4	4	11	4	5	3	12	–	–	1	1	51
Botrytis cinerea Pers.	–	–	1	1	–	–	–	–	2	–	3	5	–	–	1	1	5	2	5	12	19
Cladosporium herbarum (Pers.) Link	1	–	1	2	1	–	–	1	2	1	2	5	1	–	1	2	4	3	5	12	22
Clonostachys rosea (Link) Schroers, Samuels, Seifert	4	4	6	14	5	6	6	17	4	3	5	12	5	4	6	15	1	–	2	3	61
Chaetomium piluliferum J. Daniels	2	1	–	3	1	1	–	2	4	2	–	6	2	1	–	3	8	5	–	13	27
Epicoccum nigrum Link	–	–	1	1	–	–	1	1	–	1	–	1	–	1	–	1	2	4	2	8	12
Fusarium culmorum (W.G.Sm.) Sacc.	5	3	5	13	4	2	3	9	11	5	8	24	8	3	6	17	15	8	10	33	96
Fusarium oxysporum Schl.	11	9	5	25	10	8	5	23	12	12	7	31	11	10	6	27	20	16	10	46	152
Fusarium solani (Mart.) Sacc.	2	1	–	3	1	–	1	2	5	–	2	7	3	–	1	4	11	5	3	19	35
Humicola fuscoatra Traaen	–	–	–	–	–	–	–	–	1	1	–	2	–	–	1	1	3	4	4	11	14
Mucor racemosus Fresenius	2	1	1	4	1	1	1	3	4	2	3	9	2	1	2	5	7	3	5	15	36
Penicillium chermesinum Biourge	2	–	1	3	1	1	–	2	2	2	1	5	1	3	–	4	5	5	3	13	27
Penicillium chrysogenum Thom	–	2	2	4	1	1	1	3	3	4	2	9	1	3	1	5	6	5	4	15	36
Penicillium lividum Westling	–	–	–	–	–	–	–	–	1	–	2	3	–	–	–	–	3	–	3	6	9
Rhizoctonia solani J.G. Kühn	5	5	3	13	4	4	3	11	7	8	6	21	5	6	4	15	11	14	12	37	97
Rhizopus stolonifer (Ehrenb.) Vuill.	2	1	2	5	2	1	1	4	3	6	5	14	2	4	3	9	7	9	6	22	54
Sclerotinia sclerotiorum (Lib.) de Bary	–	2	–	2	–	–	1	1	4	3	2	9	2	1	–	3	9	6	5	20	35
Talaromyces flavus (Klöcker) Stolk & Samson	–	–	–	–	–	–	–	–	–	1	1	2	–	–	–	–	4	–	4	11	13
Trichoderma sp.	5	10	7	22	11	9	11	31	9	6	8	23	12	9	11	32	1	–	1	2	110
Total	45	42	40	127	46	40	39	125	78	63	61	202	59	51	46	156	127	97	88	312	922

population of fungi. After the application of Asahi SL, Bio-Algeen S-90, and Zaprawa Nasienna T 75 DS/WS, their population was a little more significant and was, respectively, 3.39×10^3, 4.21×10^3 and 4.30×10^3 CFU/g of soil DW. Those values, however, differed statistically from the control (Tab. 1). The available literature lacks information on the effect of the studied biostimulants on microorganism communities in the rhizosphere of scorzonera. On the other hand, these preparations, especially Beta-Chikol, proved effective in protecting scorzonera plants from infection by soil-borne pathogens [Patkowska et al. 2022]. Beta-Chikol and Asahi SL were more effective than Bio-Algeen S-90, and they considerably reduced the population of fungi regarded as pathogenic towards scorzonera, such as *Alternaria alternata*, *Alternaria scorzonerae*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* [Patkowska et al. 2022]. According to Patkowska et al. [2022], Beta-Chikol improved the emergence and health of scorzonera seedlings. Other biostimulants such as Timorex Gold 24 EC (based on tea tree oil), Biosept Active (a.s. – grapefruit extract), and Trianum P (spores of *Trichoderma harzianum* Rifai T-22) significantly increased the population of bacteria and decreased the population of fungi in the rhizospheres of carrot and scorzonera [Patkowska et al. 2020, Patkowska 2021].

A total of 922 fungi isolates were obtained from the rhizosphere of *Scorzonera hispanica.* They belonged to 16 genera (Tab. 2). The species composition of rhizospheric fungi was similar, while their quantitative composition depended on the kind of applied biostimulant and fungicide. Most isolates of fungi were obtained from the rhizosphere of control plants (312 isolates), whereas the fewest were after the application of biostimulants Asahi SL (127 isolates) and Beta-Chikol (125 isolates). Bio-Algeen S-90 and Zaprawa Nasienna T 75 DS/WS also limited the occurrence of rhizospheric fungi since 202 to 156 isolates, respectively, were obtained from those experimental combinations – all the preparations limited colonization of the rhizosphere of scorzonera by fungi considered to be pathogenic.

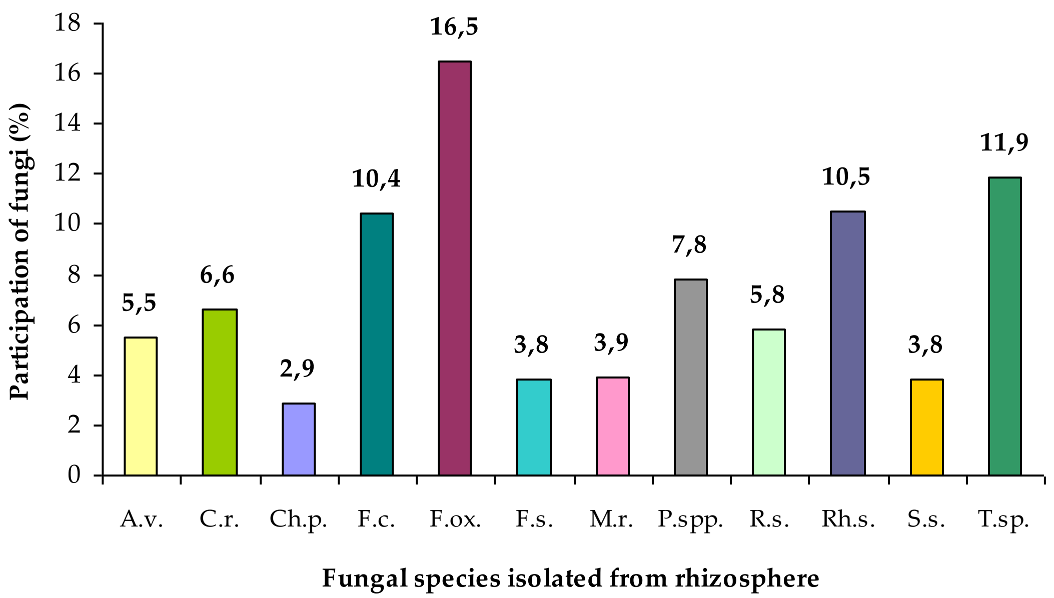


Fig. 3. Fungi most frequently isolated from the rhizosphere of scorzonera in 2014–2016 (percentage of selected fungi). Fungi species: A.v. – *Albifimbria verrucaria*, C.r. – *Clonostachys rosea*, Ch.p. – *Chaetomium piluliferum*, F.c. – *Fusarium culmorum*, F.ox. – *Fusarium oxysporum*, F.s. – *Fusarium solani*, M.r. – *Mucor racemosus*, P.spp. – *Penicillium* spp., R.s. – *Rhizopus stolonifer*, Rh.s. – *Rhizoctonia solani*, S.s. – *Sclerotinia sclerotiorum*, T.sp. – *Trichoderma* sp.

Asahi SL, Beta-Chikol, and Zaprawa Nasienna T 75 DS/WS were the most effective. The fungi that were frequently isolated from the rhizosphere belonged to the species of genera *Albifimbria*, *Clonostachys*, *Chaetomium*, *Fusarium*, *Mucor*, *Penicillium, Rhizopus, Sclerotinia*, *Rhizoctonia* and *Trichoderma*. *Fusarium oxysporum* (16.5% of all isolated fungi), *Trichoderma* sp. (11.9%), *Rhizoctonia solani* (10.5%), *Fusarium culmorum* (10.4%), *Penicillium* spp. (7.8%). Furthermore, *Clonostachys rosea* (6.6%) predominated (Fig. 3). Besides, *Rhizoctonia solani* (5.8%), *Albifimbria verrucaria* (5.5%), *Mucor racemosus* (3.9%), *Fusarium solani* (3.8%), *Sclerotinia sclerotiorum* (3.8%) and *Chaetomium piluliferum* (2.9%) were more rarely isolated from the rhizosphere (Fig. 3). Earlier studies confirmed the positive effect of other biostimulants, namely Timorex Gold 24 EC, Biosept Active and Trianum P, on limiting the occurrence of polyphagous fungi in the rhizosphere of scorzonera [Patkowska 2021]. Moreover, the same effect was shown by biostimulants Beta-Chikol, Timorex Gold 24 EC, and Trianum P in the rhizosphere of carrots [Patkowska et al. 2020]. According to Shahrajabian et al. [2021], biostimulants promote plants' growth and yield and increase their resistance to infection by pathogenic fungi and bacteria. El Hadrami et al. [2010] and Xing

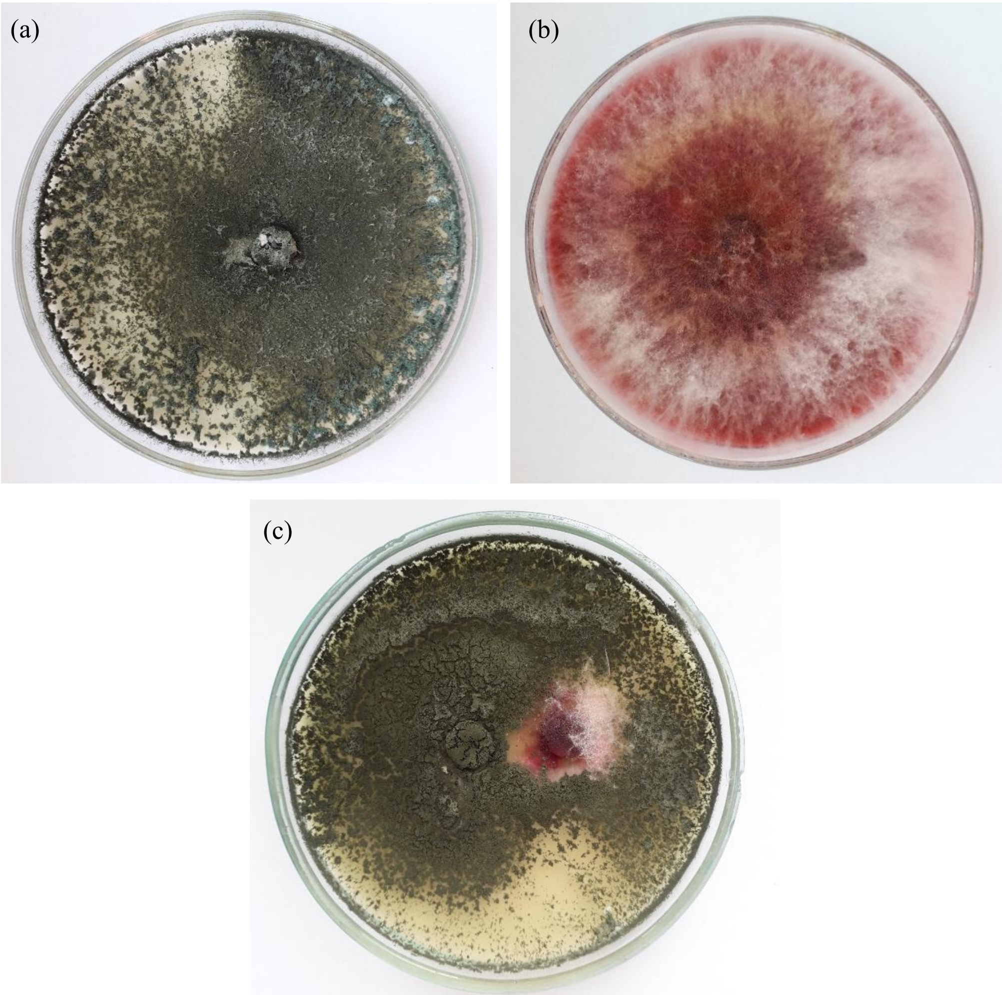
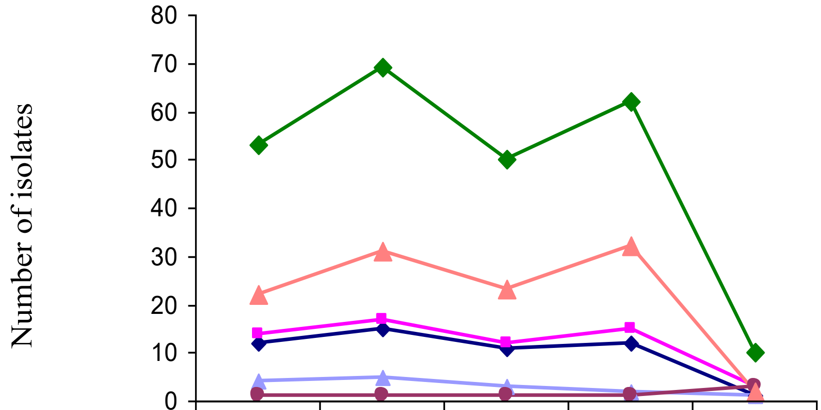


Fig. 4. 10-day-old colonies of fungi on the potato dextrose agar (PDA) medium: (a) – *Trichoderma* sp., (b) – *Fusarium culmorum*, (c) – *Fusarium culmorum* with *Trichoderma* sp.; (photo by E. Patkowska)



	A	B	C	D	E
Albifimbria verrucaria	12	15	11	12	1
Clonostachys rosea	14	17	12	15	3
Penicillium spp.	4	5	3	2	1
Epicoccum nigrum	1	1	1	1	3
Trichoderma sp.	22	31	23	32	2
Total	53	69	50	62	10

Experimental variants

Fig. 5. Number of antagonistic fungi isolated from the rhizosphere of scorzonera (sum from 2014–2016). A – Asahi SL, B – Beta-Chikol, C – Bio-Algeen S-90, D – Zaprawa Nasienna T 75 DS/WS, E – control

et al. [2015] reported that chitosan contained in Beta-Chikol effectively fights pathogenic viruses, bacteria, and fungi. As an elicitor of plant immunity, chitosan also stimulates the production of phytoalexins and callose, synthesizing proteins PR and lignins, which protects plants from infection by plant pathogens [El Hadrami et al. 2010]. Studies by other authors also confirmed the effect of biostimulants in the protection of different species of cereals and vegetables from infection by phyllosphere and soil-borne fungi, including the fungi colonizing the rhizosphere [Horoszkiewicz-Janka and Jajor 2006, Paulert et al. 2009, Jaulneau et al. 2011, Głosek-Sobieraj et al. 2019]. As reported by Głosek-Sobieraj et al. [2019], the Bio-Algeen S-90 Plus 2 biostimulant improved the health status of husked, naked oats and barley [Horoszkiewicz-Janka and Michalski 2006]. Kelpak SL containing the extract of *Ecklonia maxima* brown algae reduced the *Fusarium* foot rot in cereals and the severity of black spots (*Alternaria* spp.) in rapeseed [Horoszkiewicz-Janka and Jajor 2006]. As reported by Głosek-Sobieraj et al. [2019], the extract of green algae of the genus *Ulva* protected common bean against *Colletotrichum lindemuthianum* [Paulert et al. 2009], prevented the spread of *Blumeria graminis* in barley [Paulert et al. 2010], and the spread of powdery mildew (*Erysiphe polygoni*, *E. necator* and *Sphaerotheca fuliginea*) in bean, grapevine and cucumber [Jaulneau et al. 2011]. Biostimulants used in the present study considerably limited the polyphagous fungi population in the rhizosphere of scorzonera. This is why it should be supposed that they can effectively protect this root vegetable from infection by soil-borne fungi.

Based on laboratory tests in vitro (Fig. 4), the number of antagonistic fungi (*Clonostachys rosea*, *Albifimbria verrucaria*, *Epicoccum nigrum*, *Penicillium chermesinum*, *Penicillium chrysogenum*, *Penicillium lividum*, and *Trichoderma* sp.) towards fungi pathogenic to scorzonera (*Fusarium oxysporum*, *F. culmorum*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani*) was determined. Biostimulant Beta-Chikol and fungicide Zaprawa Nasienna T 75 DS/WS were most effective in stimulating the development of antagonistic fungi as the most antagonists were obtained from these experimental combinations (69 and 62 isolates, respectively) (Fig. 5). Asahi SL and Bio-Algeen S-90 also promoted antagonistic fungi. After the application of these biostimulants, respectively 53 and 50 isolates of antagonistic fungi were observed. The fewest antagonists occurred in the rhizosphere of control plants (10 isolates). Regardless of the applied biostimulant and fungicide, *Trichoderma* sp., *Albifimbria verrucaria*, and *Clonostachys rosea* predominated among the antagonists (Fig. 5). The antagonistic activity of those fungi consisted of antibiosis, mycoparasitism and competition [Brito et al. 2014, Masi et al. 2018, Sood et al. 2020]. Those mechanisms consist, for example, in the synthesis of siderophores, antibiotics, and lytic enzymes, especially β-1,6-glucanase and β-1,3-glucanase, which degrade the cell walls of hyphae and spores of plant pathogens [de La Cruz et al. 1992, Sood et al. 2020]. Such abilities of fungi belonging to the genus *Trichoderma* were observed towards *Rhizoctonia solani* [Roberti et al. 2015], *Alternaria alternata* [Gveroska and Ziberoski 2012], and *Sclerotinia sclerotiorum* [Smolińska and Kowalska 2018]. The present studies confirmed the high effectiveness of biostimulants in developing the population of antagonistic fungi in the rhizosphere of scorzonera. A similar effect was observed in earlier studies concerning the influence of biostimulants Beta-Chikol, Timorex Gold 24 EC, and Trianum P on the rhizosphere microorganisms of carrots [Patkowska et al. 2020].

CONCLUSIONS

1. The biostimulants, especially Asahi SL and Beta-Chikol, promoted the development of *Bacillus* sp. and *Pseudomonas* sp. populations in the rhizosphere of *Scorzonera hispanica*.

2. The fungicide and all biostimulants (especially Beta-Chikol) decreased the fungi population in the rhizosphere of scorzonera.

3. The biostimulants used to cultivate scorzonera limited the development of polyphagous fungi.

4. Independently of the applied biostimulant and fungicide, *Clonostachys rosea*, *Trichoderma* sp., and *Albifimbria verrucaria* predominated among rhizospheric antagonists.

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