

## BIODIVERSITY OF FUNGI COLONIZING SCORZONERA (*Scorzonera hispanica* L.) CULTIVATED WITH THE USE OF BIOSTIMULANTS

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### ABSTRACT

Biostimulants are friendly to the soil environment and can effectively improve the plant growth and yielding. The aim of field and laboratory studies was to establish the effect of biostimulants on the growth and on the health status of *Scorzonera hispanica* L. plants. The field experiment was carried out in south-eastern Poland on haplic luvisol. The biostimulants were applied according to the manufacturers' recommendations. Moreover, the biostimulants Asahi SL (active components: nitroguaiacolate and nitrophenolates), Beta-Chikol (a.s. – chitosan) and Bio-Algeen S90 (extract from seaweed *Ascophyllum nodosum*) were applied for the pre-sowing seed dressing of scorzonera cv. 'Duplex'. For comparison, the fungicide Zaprava Nasienna T 75 DS/WS (a.s. – tiuram 75%) was used. Untreated seeds served as control. Moreover, the biodiversity of soil-borne fungi colonizing the roots of this vegetable was determined. The number of seedlings and the health status of scorzonera plants were determined during three growing seasons. In each year of the study, both scorzonera seedlings with necrosis symptoms on the roots and the infected roots obtained after scorzonera harvest were subjected to laboratory mycological analysis. The experiments showed that, the emergence and health status of scorzonera seedlings after the application of biostimulants, especially after Beta-Chikol, were significantly better than in the control. Asahi SL and Beta-Chikol were more effective than Bio-Algeen S90 in limiting the occurrence of fungi pathogenic towards scorzonera plants. Diseased scorzonera roots were most frequently colonized by *Alternaria scorzonerae*, *Alternaria alternata*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Fusarium* spp., especially by *Fusarium oxysporum*. In conclusion, Asahi SL, Beta-Chikol and Bio-Algeen S90 can be recommended as effective biostimulants in field cultivation of *Scorzonera hispanica*.

**Key words:** high-inulin vegetable, Asahi SL, Beta-Chikol, Bio-Algeen S90, health status of plants, soil-borne pathogens

### INTRODUCTION

Plants and plant products, containing bioactive substances with a health-promoting effect, play a positive role in human nutrition [Konopiński 2003, Liu 2013, Mulero et al. 2015, Dhalaria et al. 2020]. They prevent civilization diseases, such as hypertension, diabetes, obesity, atherosclerosis, osteoporosis and cancer [Shafaghat et al. 2012, Liu 2013, Mulero et al.

2015, Dhalaria et al. 2020]. In the extremely diverse flora world, plants containing fructo-oligosaccharides, especially inulin, deserve special attention [Roberfroid 2002, Konopiński 2003, Singh and Singh 2010]. This compound, having a beneficial effect on the organisms of humans and animals, is a storage material found in tubers, bulbs and roots of plants from the families

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Asteraceae, Campanulaceae and Alliaceae (e.g. salsify, scorzonera, root chicory, artichoke, Jerusalem artichoke, platycodon, onion and garlic) [Kaur and Gupta 2002, Konopiński 2003, Maroufi et al. 2018]. Inulin is an important ingredient of dietary products; it replaces sugar and reduces fat content, thereby reducing the energy value of food intended especially for diabetics [Causey et al. 2000, Kolida et al. 2002].

Scorzonera (*Scorzonera hispanica* L.), otherwise known as serpent root takes the leading place among the plant species containing significant amounts of inulin [Konopiński 2003, Petkova 2018]. This species, belonging to the family Asteraceae, is a root vegetable, little known in many countries in the world and rarely cultivated [Konopiński 2003, Mavrodiev et al. 2004]. The edible storage root, when cooked, resembles asparagus in taste [Dolota et al. 2005]. In China, the roots of the wild-growing *Scorzonera austriaca* Willd. are used as a traditional folk panacea for many diseases [Jiang et al. 2007]. The roots of scorzonera contain inulin, polyphenolic acids, glycosides, carbohydrates, minerals and vitamins [Konopiński 2003, Dolota et al. 2005, Erden et al. 2013]. Scorzonera and its products are effective in the prevention and treatment of gastrointestinal, cardiovascular, diabetes and even cancer diseases [Causey et al. 2000, Kaur and Gupta 2002, Roberfroid 2002, Petkova 2018]. Such numerous health-promoting properties of *Scorzonera hispanica* should encourage farmers to cultivate this plant species. Profitability of the cultivation of this vegetable is determined by the size and quality of the root yield. The quality of the yield depends not only on the morphological features and chemical composition [Konopiński and Ferens 2011], but also on the plants' health condition [Patkowska and Konopiński 2008a, 2013a]. The literature provides only scarce information about the occurrence of diseases on *Scorzonera hispanica*. As reported by Choi and Thines [2015], Loerakker [1984] and Patkowska and Konopiński [2008a, 2013a], scorzonera cultivation may be threatened by fungi from genera *Alternaria*, *Fusarium*, *Rhizoctonia*, *Bremia* and *Plasmopara*. Therefore, the cultivation should consider the proper methods which limit the occurrence of plant pathogens, including soil-borne fungi.

Modern cultivation of plants, including root vegetables, make use of, for example, biostim-

ulants [Pylak et al. 2019, Ricci et al. 2019, Wadas and Dziugiel 2020]. These are the substances which facilitate the processes of the growth and development of plants. As a consequence, they increase the yield-forming potential, at the same time improving the yield quality [Du Jardin 2015]. According to Yakhin et al. [2017] a plant biostimulant is: „a formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds”. Biostimulants also include bacteria (*Arthrobacter* spp., *Bacillus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Ochrobactrum* spp., *Rhodococcus* spp.) [Vejan et al. 2016, Robledo-Buriticá et al. 2018, Seema et al. 2018, Zhao et al. 2018] and fungi (*Glomus* spp., *Claroideoglomus* spp., *Heteroconium* spp., *Trichoderma* spp.) [Colla et al. 2015, Drobek et al. 2019, Jamiołkowska et al. 2020, Patkowska et al. 2020]. They belong to the PGPM (plant growth-promoting microorganisms), they induce plants' resistance to pathogens and limit the growth and development of plant pathogens colonizing plant roots [Colla et al. 2015, Seema et al. 2018]. The main active substances used in natural preparations are fulvic and humic acids, salicylic acid, protein hydrolysates, compounds containing nitrogen, seaweed extracts, beneficial bacterial and fungal agents [Calvo et al. 2014, Drobek et al. 2019, Malik et al. 2021]. Biostimulants include, for example, Asahi SL (active components: nitroguaiacolate and nitrophenolates), Beta-Chikol (a.s. – chitosan), Bio-Algeen S90 (extract from seaweed *Ascophyllum nodosum*) and Kelpak SL (extract from seaweed *Ecklonia maxima*) [Canellas et al. 2015, Wadas and Dziugiel 2020, Malik et al. 2021]. Biostimulants are used in the cultivation of such plant species as carrot, onion, pepper, tomato, potato, barley, wheat, maize [Battacharyya et al. 2015, Begum et al. 2018, Patkowska et al. 2020, Wadas and Dziugiel 2020].

The purpose of the studies was to establish the effect of biostimulants Asahi SL, Beta-Chikol and Bio-Algeen S90 on the growth and on the health status of scorzonera (*Scorzonera hispanica* L.) plants. Moreover, the biodiversity of soil-borne fungi colonizing the roots of this vegetable was determined.

## MATERIAL AND METHODS

**Field experiment.** The experiments were carried out in 2014–2016 in south-eastern Poland (Lublin region; 51°23'N, 22°56'E), on haplic luvisol soil formed from silty medium loams. The subject of the research was scorzonera (*Scorzonera hispanica* L.) cv. 'Duplex' cultivated on ridges. The experiment involved scorzonera cultivation after winter wheat (forecrop). Disking was performed after wheat harvest, and deep ploughing before winter (about 25 cm). Before scorzonera sowing, the soil contained 1.06–1.15% of humus in the 0–20 cm depth and was characterized by slightly acidic (pH in 1 M KCl – 5.76–5.90). The amount of available phosphorus, potassium, and magnesium was as follows: P – 146.8; K – 111.5; Mg – 102.9 mg·kg<sup>-1</sup> soil. NPK mineral fertilization was applied in the spring in the amount of: 100:50:100 kg·ha<sup>-1</sup>. Cultivator treatment and harrowing was performed after the application of mineral fertilizers. The experiment was set up as a completely randomized block design in 4 replicates. The area of each experimental plot was 14 m<sup>2</sup>. The experiment was established in the first 10-day period of May. Scorzonera seeds were sown to a depth of 3 cm, in rows every 50 cm, in the amount of 12 kg·ha<sup>-1</sup>. The plants were harvested in the second half of October.

Biostimulants and a fungicide were used for pre-sowing treatment of scorzonera seeds. These were the following preparations: Asahi SL (active components: 0.1% sodium 5-nitroguaiacolate, 0.2% sodium ortho-nitrophenolate, and 0.3% sodium para-nitrophenolate) produced by Arysta/UPL Polska sp. z o.o.; Beta-Chikol (a.s. – chitosan) produced by Poli-Farm, Łowicz, Poland; Bio-Algeen S90 produced by Schulze & Hermsen GmbH, Germany. Bio-algeen S90 is an extract from *Ascophyllum nodosum* which contains amino acids, vitamins, alginic acids and other active components of seaweeds, as well as macronutrients (N, K, P, Mg, Ca) and micronutrients (Fe, B, Zn, Cu, Mn, Co, Se). For comparison, the fungicide Zaprawa Nasienna T 75 DS/WS (a.s. – tiuram 75%) produced by Organika-Azot in Jaworzno, Poland was used. Untreated seeds served as control. The preparations were applied according to the manufacturers' recommendations: Asahi SL – 50 ml·kg<sup>-1</sup> seeds, Beta-Chikol – 100 ml·kg<sup>-1</sup> seeds, Bio-Algeen S90 – 15 ml·kg<sup>-1</sup> seeds,

Zaprawa Nasienna T 75 DS/WS – 5 g·kg<sup>-1</sup> seeds. The second protective treatment was performed at the beginning of leaf development stage (BBCH 10–11 according to the scale of Biologische Bundesanstalt, Bundessortenamt and Chemical Industry).

**The health status of plants.** In each growing season, the emergence and percentage of diseased scorzonera seedlings were determined in individual experimental treatments. The health of scorzonera seedlings was evaluated at the stage of 4–5 leaves (BBCH 14–15) in each year of the study. According to the method described by Patkowska et al. [2020] for carrot, 50 scorzonera seedlings were randomly selected from each plot. The level of infection was determined according to a five-score rating scale for carrot (where 0° – no disease symptoms, 1° – necrosis up to 10% of the root surface, 2° – necrosis up to 25% of the root surface, 3° – necrosis up to 50% of the root surface, and 4° – over 50% of the root area infected) [Patkowska et al. 2020]. The disease index was calculated according to McKinney's formula [Mielniczuk et al. 2020]:

$$\text{Disease index} = \frac{\sum (a_i \times b_i)}{n \times c} \times 100$$

where:  $a_i$  – score of rating scale (from 0° to 4°),  $b_i$  – number of roots in a given score of the rating scale;  $n$  – total number of roots observed;  $c$  – highest score of the rating scale.

In each year of the study, 40 seedlings (BBCH 14–15) with disease symptoms (Phot. 1) were collected from particular experimental treatments for mycological analysis of the infected roots. Additionally, after the harvest (second decade of October), 40 randomly selected scorzonera roots (BBCH 49) from each experimental treatment with necrotic and etiological signs were subject to mycological analysis.

**Mycological analysis of plants.** According to the method described by Patkowska [2020] for carrot, the infected scorzonera roots were rinsed for 30 min under running tap water, subsequently disinfected in 1% sodium hypochlorite. Surface-disinfected plant material was rinsed three times for three minutes in sterile distilled water. Three-millimeter fragments were cut from the thus prepared plant material and placed in 9 cm sterile Petri dishes on SNA (selective nutrient agar) medium with the following composition:



**Phot. 1.** Six-week-old seedlings of scorzonera (photo by E. Patkowska)

38 g saccharose, 0.7 g  $\text{NH}_4\text{NO}_3$ , 0.3 g  $\text{KH}_2\text{PO}_4$ , 0.3 g  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ , 20 g agar and trace quantities of  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \times 7\text{H}_2\text{O}$  and  $\text{MnSO}_4 \times 5\text{H}_2\text{O}$ . In each of the experimental variants, 100 fragments of infected scorzonera roots were examined. After 10–12 days, fungal cultures were transferred to sterile Petri dishes with PDA (potato dextrose agar) medium and incubated at 20–22°C, with 12 h light/12 h dark cycles. After 14–24 days, fungal colonies were identified to the genus and species level (morphological structures: mycelium, conidiophores and conidia) under a microscope, based on the keys and monographs listed by Patkowska et al. [2020]. Moreover, SNA and PDA media were used for *Fusarium* sp. [Leslie and Summerell 2006]. Fungi of the genus *Penicillium* were identified on Czapek-Dox and Malt media [Ramirez 1982]. The number and percentage of occurrence of the recovered fungal species were calculated. Mycological analysis allowed to determine

the quantitative and qualitative composition of the fungi colonizing scorzonera roots.

**Statistical analysis.** Results concerning the density of scorzonera plants, health status and scorzonera disease index were statistically analyzed. The means were compared to the use of the least significant differences based on the Tukey's test ( $p \leq 0.05$ ). Statistical calculations were carried out using Statistica, version 7.1 (StatSoft, Krakow, Poland).

## RESULTS AND DISCUSSION

Biostimulants Asahi SL, Beta-Chikol, and Bio-Algeen S90 used in the field experiment improved seed germination, emergence and root health of scorzonera in comparison with the control plants. The density of scorzonera plants in particular seasons of vegetation ranged from 29 (control) to 60 plants  $\cdot \text{m}^{-2}$  (Tab. 1). Depending on the experimental treatments, the av-

erage number of seedlings ranged from 34 (control) to 56.6 plants·m<sup>-2</sup>. The best emergences were found after the application of Beta-Chikol (56.6 plants·m<sup>-2</sup> on average) and Asahi SL (54.3 on average). A slightly lower average density of seedlings was found after the application of biostimulant Bio-Algeen S90 (48) and fungicide Zaprawa Nasienna T 75 DS/WS (45.6). The fewest scorzonera seedlings grew on control plots (34 on average). Other studies confirmed the stimulating effect of pre-sowing seed treatment with Beta-Chikol (chitosan) on emergence, growth and health status of plants from the family Fabaceae (such as runner bean, pea and soybean) [Patkowska 2005, Pięta et al. 2005, Patkowska and Krawiec 2016]. Chitosan stimulated seed germination and growth of carrot plants [Patkowska et al. 2020] and Bio-Algeen S90 (seaweed *Ascophyllum nodosum*) growth of potato plants [Wadas and Dziugiel 2020]. Asahi SL, a biostimulator based on nitro-phenolic compounds, stimulated seed germination, seedling growth and the increase of the

biomass and yield of rape, maize, bean, soybean, pepper and tomato [Kocira 2017].

Biostimulants and a fungicide significantly reduced the occurrence of diseased scorzonera plants. The proportion of seedlings with disease symptoms ranged from 2% to 12.5% in individual years of the study (Tab. 1). The highest number of infected seedlings was found in control (10.6% on average), and the lowest after the application of Beta-Chikol (3.2% on average) and Zaprawa Nasienna T 75 DS/WS (3.6%). The mean proportion of diseased seedlings after Asahi SL (6.2%) and Bio-Algeen S90 (7.2%) application was slightly higher, but differed significantly from control (10.6%). A positive effect of biostimulators, especially of chitosan (Beta-Chikol), on the growth and healthiness of seedlings and older plants of carrot was confirmed by Patkowska et al. [2020].

The indicator of the protective effect of the applied biostimulants against plant infection by soil-borne pathogens was the value of the disease index of scor-

**Table 1.** Number of plants per 1 m<sup>2</sup> and percentage of diseased scorzonera seedlings

Experimental treatment	Field stand per 1 m <sup>2</sup>				Diseased seedlings (%)			
	2014	2015	2016	mean	2014	2015	2016	mean
Control	35.0 c	29.0 c	38.0 c	34.0 c	10.0 a	9.5 a	12.5 a	10.6 a
Asahi SL	54.0 a	50.0 a	59.0 a	54.3 a	5.5 b	5.0 b	8.0 b	6.2 b
Beta-Chikol	58.0 a	52.0 a	60.0 a	56.6 a	3.0 c	2.0 c	4.5 c	3.2 c
Bio-Algeen S90	47.0 b	46.0 b	51.0 b	48.0 b	6.5 b	6.5 b	8.5 b	7.2 b
Zaprawa Nasienna T 75 DS/WS	45.0 b	44.0 b	48.0 b	45.6 b	3.5 c	2.5 c	5.0 c	3.6 c

Values in columns marked with the same letter do not differ significantly at  $p \leq 0.05$ .

**Table 2.** Values of the disease index of scorzonera seedlings in each year of studies

Experimental treatment	Disease index			
	2014	2015	2016	mean
Control	19.6 a	24.6 a	25.0 a	23.1 a
Asahi SL	12.2 b	15.0 b	17.2 b	14.8 b
Beta-Chikol	8.4 c	10.2 c	12.6 c	10.4 c
Bio-Algeen S90	14.3 b	17.6 b	19.4 b	17.1 b
Zaprawa Nasienna T 75 DS/WS	9.0 c	11.4 c	13.3 c	11.2 c

Values in columns marked with the same letter do not differ significantly at  $p \leq 0.05$ .

zonera seedlings. The disease index of seedling roots in particular experimental treatments ranged from 8.4 to 25 (Tab. 2). The lowest values of the disease index were recorded after the application of Zaprawa Nasienna T 75 DS/WS (11.2 on average) and Beta-Chikol (10.4 on average). Asahi SL and Bio-Algeen S90 were slightly less effective in protecting the seedlings against infection by soil-borne pathogens, because the disease index was higher (14.8 and 17.1, respectively). The highest value of the disease index was found for control (23.1 on average). After the application of chitosan (Beta-Chikol) considerably lower values of the disease index of seedlings were also recorded in the field cultivation of carrot as compared to the control [Patkowska et al. 2020]. The health status of plants, their resistance to infectious factors and the yielding are also related to the content of chlorophyll in the leaves [ALKahtani et al. 2020, Wadas and Dziugiel 2020]. Seaweed *Ascophyllum nodosum* extracts (Bio-Algeen S90) applied to soil or on foliage caused an increase in the leaf chlorophyll content of wheat, barley, maize, bean, pepper, tomato, and strawberry [Battacharyya et al. 2015, Begum et al. 2018, Wadas and Dziugiel 2020]. Foliar application of seaweed extracts *Ecklonia maxima* and *Ascophyllum nodosum* increased potato yield [Haider et al. 2012, Wierzbowska et al. 2015, Wadas and Dziugiel 2020]. Biostimulants based on seaweed extracts improved plant growth and yield of carrot, onion, pepper, tomato, potato, barley, wheat, maize [Sharma et al. 2014, Battacharyya et al. 2015, Begum et al. 2018, Patkowska et al. 2020, Wadas and Dziugiel 2020].

As a result of mycological analysis, 674 colonies of fungi belonging to 8 genera were isolated from infected roots of scorzonera seedlings (Tab. 3). The highest population of fungi was isolated from control seedlings (263 isolates). The applied preparations limited the root infection by soil-borne fungi. Beta-Chikol, Asahi SL and Zaprawa Nasienna T 75 DS/WS proved to be most effective. After the application of those biostimulators, 85, 91, and 97 fungal isolates, respectively, were obtained from scorzonera seedlings. A slightly smaller protective effect against the root infection by fungi was shown by Bio-Algeen S90. 138 fungal isolates were obtained after the application of this biostimulator. Diseased scorzonera seedlings were colonized mainly by *Fusarium oxysporum* (31%

all isolates) and *Rhizoctonia solani* (17.8%) – Figure 1A. Seedling roots were less colonized by *Alternaria scorzonerae* (7%), *Fusarium solani* (4.2%), *Fusarium sporotrichioides* (4.2%), *Fusarium graminearum* (3.7%), and saprotrophic fungi such as *Trichoderma* sp. (4.6%), *Clonostachys rosea* (4%) and *Penicillium janczewskii* (9%), which are considered pathogenic. Earlier studies demonstrated that biostimulants, especially Beta-Chikol and Trianum P (*Trichoderma harzianum* T-22) considerably improved the health status of carrot seedlings [Patkowska et al. 2020].

After harvest, 844 colonies of fungi belonging to 14 genera were obtained from scorzonera roots (Tab. 4). Like in case of seedlings, the biggest population of fungi was isolated from the roots of control plants (348 isolates). Beta-Chikol and Asahi SL were most effective in reducing the infection of scorzonera roots by fungi (97 and 107 isolates, respectively). The application of Bio-Algeen S90 and Zaprawa Nasienna T 75 DS/WS limited the occurrence of soil-borne fungi on the examined scorzonera parts to a smaller extent (172 and 121 isolates, respectively). After harvest, *Fusarium culmorum*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* were most frequently isolated from diseased roots, and their percentage was 9.8%, 11.7%, 19.4% and 26.1%, respectively (Fig. 1B). Additionally, *Alternaria scorzonerae* (5.7%), *Alternaria alternata* (5.3%), *Fusarium solani* (2.5%) and saprotrophic fungi *Clonostachys rosea* (2.8%), *Trichoderma* sp. (4.6%) and *Penicillium janczewskii* (4.4%) were less numerous. Moreover, fungi of the genera *Acremonium*, *Botrytis*, *Cladosporium*, *Cylindrocarpon*, *Rhizopus* and *Sarocladium* were identified (Tab. 4). Polyphages listed above are common pathogens of various root vegetables (parsley, carrot, celery, red beet) [Nawrocki 2005, Mazur and Nawrocki 2007, Smolińska and Kowalska 2018, Patkowska 2020]. According to Lima et al. [2016], *Alternaria alternata* and *Alternaria dauci* were frequently isolated from carrot seeds and seedlings. Fungi of the genera *Alternaria*, *Fusarium*, *Sclerotinia*, *Rhizoctonia* were also shown to infect roots of high-inulin vegetables such as salsify [Patkowska and Konopiński 2008b, 2011], root chicory [Patkowska and Konopiński 2013b] and scorzonera [Patkowska and Konopiński 2008a, 2013a]. According to Loerakker [1984], the emergence and yield of scorzonera were considerably

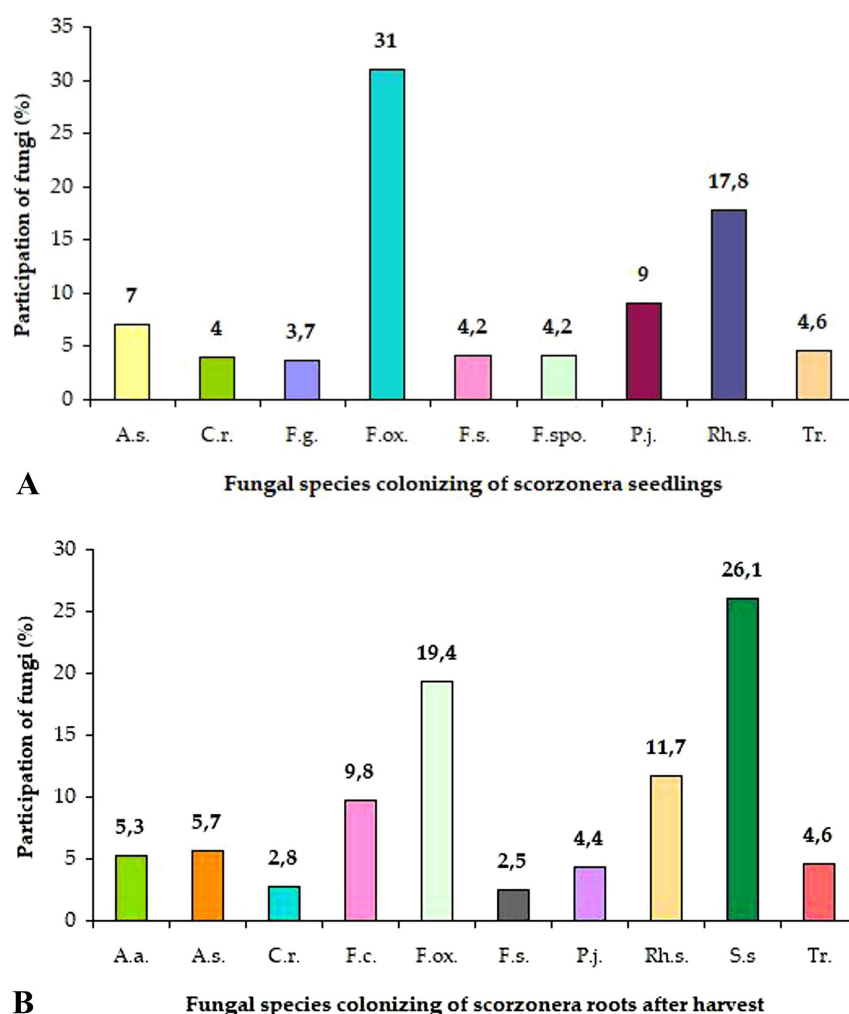
**Table 3.** Fungi isolated from diseased scorzonera seedlings (number of isolates from 2014–2016)

Fungus species	Experimental treatment / Fungi isolated from diseased scorzonera seedlings (number of isolates)																				sum of isolates
	Asahi SL				Beta-Chikol				Bio-Algeen S90				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	
<i>Albifimbria verrucaria</i> (Alb. & Schwein.) L. Lombard & Crous	3	3	2	8	4	3	3	10	1	1	–	2	1	1	–	2	–	–	–	–	22
<i>Alternaria scorzonerae</i> (Aderh.) Loer.	1	2	1	4	–	1	2	3	2	5	4	11	–	2	3	5	5	9	10	24	47
<i>Cladosporium cladosporioides</i> (Fres) de Vries	1	–	–	1	–	–	–	–	1	1	–	2	–	1	–	1	4	6	3	13	17
<i>Clonostachys rosea</i> (Link) Schroers, Samuels, Seifert	2	2	1	5	3	2	4	9	3	2	2	7	2	1	3	6	–	–	–	–	27
<i>Fusarium culmorum</i> (Wm.G. Sm.) Sacc.	–	–	1	1	–	–	–	–	–	1	1	2	–	–	–	–	4	6	5	15	18
<i>Fusarium graminearum</i> Schwabe	1	–	–	1	–	–	–	–	1	1	1	3	–	1	1	2	5	8	6	19	25
<i>Fusarium oxysporum</i> Schl.	11	9	10	30	10	8	6	24	15	13	14	42	12	10	11	33	24	27	29	80	209
<i>Fusarium solani</i> (Mart.) Sacc.	–	–	–	–	–	–	–	–	2	1	1	4	–	–	–	–	9	8	7	24	28
<i>Fusarium sporotrichioides</i> Sherb.	–	1	1	2	–	–	1	1	4	1	–	5	2	1	–	3	8	5	4	17	28
<i>Penicillium janczewskii</i> K.W. Zaleski	3	2	3	8	2	1	2	5	6	5	4	15	4	3	2	9	9	8	7	24	61
<i>Rhizoctonia solani</i> J.G. Kühn	5	6	5	16	4	5	5	14	8	10	7	25	6	7	5	18	15	18	14	47	120
<i>Trichoderma</i> sp.	5	5	5	15	7	5	7	19	2	11	7	20	5	7	6	18	–	–	–	–	31
Sum of isolates	32	30	29	91	30	25	30	85	45	52	41	138	32	34	31	97	83	95	85	263	674

**Table 4.** Fungi isolated from diseased scorzonera roots after harvest (number of isolates from 2014–2016)

Fungus species	Experimental treatment / Fungi isolated from diseased scorzonera roots after harvest (number of isolates)																				Sum of isolates
	Asahi SL				Beta–Chikol				Bio–Algeen S90				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	
<i>Acremonium rutilum</i> W. Gams	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	2	3	2	7	8
<i>Alternaria alternata</i> (Fr.) Keissler	–	3	2	5	2	–	1	3	6	4	–	10	4	2	–	6	11	7	3	21	45
<i>Alternaria scorzonerae</i> (Aderh.) Loer.	2	2	2	6	3	1	1	5	5	6	–	11	3	4	–	7	8	9	2	19	48
<i>Botrytis cinerea</i> Pers.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	3	4	9	9
<i>Cladosporium herbarum</i> (Pers.) Link	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	4	5	3	12	13
<i>Cylindrocarpon didymum</i> (Harting) Wollenw.	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	4	3	2	9	10
<i>Clonostachys rosea</i> (Link) Schroers, Samuels, Seifert	3	2	2	7	4	2	3	9	3	–	–	3	3	2	–	5	–	–	–	–	24
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	5	3	3	11	4	2	2	8	9	6	4	19	6	4	3	13	13	11	8	32	83
<i>Fusarium oxysporum</i> Schl.	10	7	7	24	9	6	5	20	14	12	10	36	11	10	8	29	19	18	18	55	164
<i>Fusarium solani</i> (Mart.) Sacc.	–	–	–	–	–	–	–	–	–	1	1	2	–	–	–	–	6	7	6	19	21
<i>Mucor hiemalis</i> Wehmer	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	2	2
<i>Penicillium janczewskii</i> K.W. Zaleski	2	–	1	3	1	1	–	2	5	2	1	8	2	1	–	3	10	6	5	21	37
<i>Penicillium chrysogenum</i> Thom	–	–	–	–	–	–	–	–	2	–	–	2	–	–	–	–	5	3	–	8	10
<i>Rhizoctonia solani</i> J.G. Kühn	6	5	3	14	6	5	2	13	9	7	6	22	6	4	4	14	12	14	10	36	99
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	3	–	7	7
<i>Sarocladium kiliense</i> (Grütz) Summerb.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	3	1	5	5
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	13	8	8	29	11	7	6	24	19	14	12	45	15	11	10	36	32	28	26	86	220
<i>Trichoderma</i> sp.	3	3	2	8	4	5	3	12	5	4	2	11	4	3	1	8	–	–	–	–	39
Sum of isolates	44	33	30	107	44	29	23	96	78	57	37	172	54	41	26	121	135	123	90	348	844





**Fig. 1.** Fungi most frequently isolated from scorzonera plants in 2014–2016: (A) – percentage of selected fungi isolated from scorzonera seedlings; (B) – percentage of selected fungi isolated from scorzonera roots after harvest.

Fungi species: A.a. – *Alternaria alternata*, A.s. – *Alternaria scorzonerae*, C.r. – *Clonostachys rosea*, F.c. – *Fusarium culmorum*, F.g. – *Fusarium graminearum*, F.ox. – *Fusarium oxysporum*, F.s. – *Fusarium solani*, F.spo. – *Fusarium sporotrichioides*, P.j. – *Penicillium janczewskii*, sRh.s. – *Rhizoctonia solani*, S.s. – *Sclerotinia sclerotiorum*, Tr. – *Trichoderma* sp.

lower as a result of *Alternaria scorzonerae* infection. Pathogenicity tests carried out in the growth chamber confirmed high harmfulness of *Alternaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Pythium irregulare* and *Rhizoctonia solani* for scorzonera seeds and seedlings [Patkowska and Konopiński 2008a].

As reported by Koziara et al. [2006] and Yakhin et al. [2017], many of biostimulants, especially Beta-Chi-

kol, Asahi SL and Bio-Algeen S90, not only mitigate stress-induced limitations and regulate/modify physiological processes in plants to stimulate growth and increase productivity, but also directly limit the development of phytopathogens. In in vitro conditions, Asahi SL effectively inhibited the growth and development of *Fusarium avenaceum*, *F. culmorum*, *F. oxysporum* and *F. graminearum* [Ogórek et al. 2011]. Moreover,

this biostimulator limited the infection of winter rape plants by *Sclerotinia sclerotiorum*, *Leptosphaeria maculans*, *Alternaria alternata* [Pusz and Płaskowska 2008] and potato tubers by *Streptomyces scabies* [Sawicka and Krochmal-Marczak 2009]. Chitosan (Biochikol 020 PC) protected *Pisum sativum* against *Fusarium culmorum*, *F. oxysporum*, *F. avenaceum*, *Boeremia exigua*, *Alternaria alternata*, *Haematonectria haematococca*, *Peyronellaea pinodes* and *Thanatephorus cucumeris* [Patkowska and Krawiec 2016]. Chitin (chitosan) is known as a strong fungal microbe-associated molecular pattern molecule, which is recognized by plants and which activates their immune response [Gai et al. 2019]. Bio-Algeen S90 protected the potato tubers against *Pectobacterium carotovorum* subsp. *carotovorum*, *Dickeya* spp., *Phytophthora infestans* and *Fusarium* spp. [Głosek-Sobieraj et al. 2019].

The present studies confirmed the positive effect of biostimulators on the health status of *Scorzonera hispanica*, thus confirming the possibility of applying them in the field cultivation of this species. They considerably limited root colonization by polyphagous soil-borne fungi. According to Kocira [2017], biostimulators do not only stimulate the growth and development of plants and affect metabolic processes occurring in the plant but they also increase plants' resistance to stress factors, including plant pathogens. Moreover, being safe to people and the environment, they find application in protecting plants from pathogenic bacteria and fungi.

## CONCLUSIONS

1. The biostimulants used in the field experiment improved seed germination and emergence of *Scorzonera hispanica*.

2. The health status of scorzonera plants was differentiated and it depended on the type of biostimulant.

3. The biostimulants limited scorzonera roots colonization by polyphagous fungi.

4. Asahi SL and Beta-Chikol were more effective than Bio-Algeen S90 in limiting the occurrence of fungi pathogenic towards scorzonera plants.

5. Diseased scorzonera roots were most frequently colonized by *Alternaria scorzonerae*, *A. alternata*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Fusarium* spp., especially by *Fusarium oxysporum*.

## SOURCE OF FUNDING

The sources of funding: Financed by the „Excellent Science” program of the Minister of Education and Science of Republic of Poland.

PL: Dofinansowano z programu „Doskonała nauka” Ministra Edukacji i Nauki.

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