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The Influence of *Trichoderma harzianum* Rifai T-22 and Other Biostimulants on Rhizosphere Beneficial Microorganisms of Carrot

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Received: 30 September 2020; Accepted: 22 October 2020; Published: 23 October 2020

Abstract: The principles of good agricultural and horticultural practice, which consider both giving environmental protection and high yielding of plants, require modern cultivation methods. Modern cultivation of horticultural plants uses, for example, cover crops, living mulches, plant growth-promoting microorganisms (PGPMs), plant growth regulators (PGRs) and other biostimulants protecting the soil against degradation and plants against phytopathogens and stress. The purpose of field and laboratory studies was to determine the effect of Trianum P (containing *Trichoderma harzianum* Rifai T-22 spores), Beta-Chikol (a.s.—chitosan), Timorex Gold 24 EC (based on tea tree oil) and fungicide Zaprawa Nasienna T 75 DS/WS (a.s.—tiuram 75%) on the health of carrot (*Daucus carota* L.) plants and the microorganism population in the rhizosphere of this plant. Moreover, the antagonistic effect of rhizosphere fungi on selected carrot fungal pathogens was determined. Laboratory mycological analysis allowed one to determine the qualitative and quantitative composition of fungi colonizing the underground parts of carrot plants. In addition, the total population of fungi and bacteria was determined (including *Bacillus* sp. and *Pseudomonas* sp.) based on the microbiological analysis of the rhizosphere soil. The application of the plant growth-promoting fungus (*Trichoderma harzianum* T-22), chitosan and tea tree oil positively influenced the growth, development and health status of carrot plants. *T. harzianum* T-22, chitosan and fungicide most effectively protected carrots against infection by soil-borne fungi from the genus *Alternaria*, *Fusarium*, *Haematonectria*, *Sclerotinia* and *Rhizoctonia*. The rhizosphere population of *Bacillus* sp. and *Pseudomonas* sp. in the treatments with Trianum P or Zaprawa Nasienna T 75 DS/WS was bigger than in the other experimental treatments. A reverse relationship was observed in the population of rhizosphere fungi. *T. harzianum* T-22, chitosan and tea tree oil promoted the growth of antagonistic fungi (*Albifimbria* sp., *Clonostachys* sp., *Penicillium* sp., *Talaromyces* sp. and *Trichoderma* sp.) in the carrot rhizosphere. Antagonistic activity of these fungi towards *Alternaria dauci*, *Alternaria radicina*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* was higher after the application of the preparations compared to control. Consequently, Trianum P, Beta-Chikol and Timorex Gold 24 EC can be recommended as plant biostimulants in ecological agricultural production, including *Daucus carota* cultivation.

Keywords: PGPMs (plant growth-promoting microorganisms); chitosan; tee tree oil; plant biostimulants; soil-borne phytopathogens; antagonistic fungi; biocontrol; biotic effect; crop production

1. Introduction

Carrot (*Daucus carota* L.) belongs to the family Apiaceae. It is one of the most popular vegetables, and has great economic importance worldwide. The leading producers of carrots are the United States, China, Uzbekistan and the Russian Federation [1,2]. Carrot roots are rich in beta-carotene (vitamin A precursor), and also contain vitamin K, vitamin E (alpha-tocopherol), vitamin C, calcium, magnesium, potassium, phosphorus, other vitamins and minerals, phenolic compounds, flavonoids and dietary fiber [3–5]. This vegetable has many healthy properties: it exerts antioxidation and anticancer effects, strengthens the immune system, lowers cholesterol blood levels, prevents premature ageing and has a positive influence on eyesight, skin, nails and hair [3,6]. It should be one of the basic vegetables in the human diet. Therefore, in carrot cultivation, healthy, high-quality seeds and roots should be obtained, without any residues of pesticides, heavy metals or mycotoxins harmful to human and animal health. Such effects are provided by ecological cultivations based on biological protection [1,7–14]. They reduce the number of chemical protection agents, while limiting plant infection by phytopathogens, including toxigenic fungi (*Alternaria* sp., *Fusarium* sp. and *Penicillium* sp.) [9,15–19].

Daucus carota can be infected by a number of plant pathogens, including viruses, bacteria and fungi [1,20–26]. Adams et al. [20] have reported that carrot plants can be infected by the following viruses: CMoV (carrot mottle virus), CYLV (carrot yellow leaf virus), PYFV (parsnip yellow fleck virus), CtRLV (carrot red leaf virus) and PYFV (parsnip yellow fleck virus). As reported by Nesha and Siddiqui [21], health of this plant was reduced by *Pectobacterium carotovorum* pv. *carotovorum* (Jones) Waldee, causing bacterial soft rot and *Xanthomonas campestris* pv. *carotae* (Pammel) Dowson, causing bacterial leaf blight. Lerat et al. [22] informed about *Streptomyces scabies* Lambert and Loria, causing common scab on carrot, while Rachamallu [3] reported on *Agrobacterium rhizogenes* Conn (*Rhizobium rhizogenes*), causing hairy roots.

Fungal diseases are among the key biotic factors responsible for carrot yield. The major fungal diseases affecting carrot that can cause significant crop losses include alternariosis (*Alternaria dauci* (Kühn)) Groves and Skolko, *Alternaria radicina* Meier, Drechsler and Eddy), fusariosis (*Fusarium* spp.), gray mold (*Botrytis cinerea* Pers.), rhizoctoniosis (*Rhizoctonia solani* J.G. Kühn), white mold (*Sclerotinia sclerotiorum* (Lib.)) de Bary) and cercospora leaf spot (*Cercospora carotae* (Pass.) Solheim) [24–36]. According to Boiteux et al. [37] and Naqvi [38], powdery mildew (*Erysiphe heraclei* DC.) and downy mildew (*Peronospora crustosa* (Fr.) Fr.) can also occur on carrot leaves.

Modern plant protection is based on the sustainable use of pesticides, mainly the application of non-chemical methods of plant protection against pests, diseases and weeds [16]. The organic production system uses biological and physiological plant mechanisms supported by the rational use of conventional, biological and natural preparations [8,10,11,16,39,40]. Moreover, the principles of good agricultural and horticultural practice, taking into account both environmental protection and high yielding of plants, require modern cultivation methods. Modern cultivation of horticultural plants, including carrots, applies cover crops, living mulches, PGPMs (plant growth-promoting microorganisms) and PGRs (plant growth regulators) protecting the soil against degradation and plants from phytopathogens and stress [14,41–47].

PGPMs are groups of rhizosphere microorganisms capable of colonizing the root environment [47–49]. Some of the microbes that inhabit this zone are bacteria and fungi that are able to efficiently colonize the roots and rhizosphere soil [48,49]. The group of PGPFs (plant growth-promoting fungi) also includes *Trichoderma* sp.; some groups of *Trichoderma* species are associated with plant roots, where they either form a symbiotic relationship or occur as plant endophytes [50,51]. However, *Trichoderma* rhizosphere-competent strains have been shown to exert direct effects on plants, by increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination and stimulating plant defenses against biotic and abiotic damage [52]. *Trichoderma* spp. can improve the health status of plants by inducing systemic resistance (ISR) [53,54]. Plant resistance is associated with the formation of specific PR proteins (pathogenesis-related proteins) toxic to many pathogens such as *Botrytis cinerea, Fusarium culmorum, F. oxysporum, Rhizoctonia solani* or *Phytophthora infestans* [55]. These proteins inhibit the formation and germination of fungal spores, strengthen the host's cell walls and degrade the cell walls of plant pathogens [55].

Trichoderma is the most commonly used biological control agent of plant pathogens and has long been known as an effective antagonist of plant pathogenic fungi [49,56–58]. The antagonistic activities of *Trichoderma* towards plant pathogens are a combination of several mechanisms, including nutrient and/or space competition, antibiosis associated with the secretion of antibiotic and direct mycoparasitism, which involves the production of cell wall-degrading enzymes [49,50,59]. *Trichoderma harzianum* exhibits specific antagonistic properties [56,58,60,61]. A considerable interest in *Trichoderma* properties and the possibility of using them in agriculture led to the development of commercial products using various species of *Trichoderma* [62,63]. One such product is Trianum P, which contains the spores of *T. harzianum* T-22.

Biostimulants that alleviate the effects associated with the occurrence of abiotic and biotic stresses include, among others, Beta-Chikol (a.s.—chitosan) and Timorex Gold 24 EC (based on tea tree oil) are based on natural components. Chitosan or chitin is a natural polysaccharide consisting of two D-glucosamine molecules and naturally present in the cell walls of fungi, crustaceans and insect exoskeleton [47,64,65]. The organic compound is obtained by chitin distillation through the action of concentrated sodium hydroxide at elevated temperature or using enzymes [66]. It has antiviral, antibacterial and antifungal properties [67,68]. Chitosan, as an elicitor of plant resistance, stimulates the formation of phytoalexins and callose, synthesis of PR proteins and lignin [68]. Natural tea tree oil is obtained from the leaves and small branches of *Melaleuca alternifolia* L. It contains mainly terpenes (p-cymene, terpinolene, terpinen-4-ol and 1,8-cineole), sesquiterpenes and their respective alcohol (monoterpene alcohol-terpineol) [69,70]. It has a strong antiseptic effect by destroying cell membranes and organelles [71–73]. It is used in biological plant protection against bacterial and fungal pathogens [15,72–74].

The purpose of the study was to determine the effect of *Trichoderma harzianum* T-22, chitosan and tea tree oil on the health status of carrot plants and microorganism population in the rhizosphere. Moreover, the antagonistic activity of rhizosphere fungi towards selected fungal pathogens of carrot was determined.

2. Materials and Methods

2.1. Field Trials

Carrots (*Daucus carota* L.) were grown for three growing seasons (2014–2016), in South-Eastern Poland (Lublin region; 51°23′ N, 22°56′ E, World Reference Base for Soil Resources (WRB): Haplic Luvisol formed from silty medium loams). The subjects of the research were plants and rhizosphere soil of the carrot cv. 'Flakkese 2'. The experiment was set up as a completely randomized block design in 4 replicates. The area of each plot was 33 m^2. Mineral fertilization was applied in the spring at the following amounts of NPK: 150:50:160 kg/ha. Carrot was sown in the first 10-day period of May in rows (spacing—50 cm); the seeding rate was 2.6 kg/ha.

Before sowing, carrot seeds were dressed with the following preparations (biostimulants): Trianum P (containing *Trichoderma harzianum* Rifai T-22) produced by Koppert BV, Veilingweg, Netherlands; Beta-Chikol (a.s.—chitosan) produced by Poli-Farm, Łowicz, Poland; Timorex Gold 24 EC (based on essential tea tree oil) produced by Biomor Israel Ltd., Katzerin, Israel. 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 a control. The preparations were applied according to the manufacturers' recommendations: Beta-Chikol—100 mL/kg seeds, Timorex Gold 24 EC—150 mL/kg seeds, Trianum P—50 g/kg seeds and Zaprawa Nasienna T 75 DS/WS—5 g/kg seeds. The second protective treatment was performed at the beginning of the 2-leaf stage (BBCH 12 according to the scale of Biologische Bundesanstalt, Bundessortenamt and Chemical Industry).

In each growing season, the emergence and percentage of diseased carrot seedlings were determined in individual experimental treatments. The health of carrot seedlings was evaluated at the stage of 3-4 leaves (BBCH 13-14) in each year of the study. Fifty seedlings were randomly selected from each plot. The level of infection was determined according to a five-score rating scale for scorzonera (where 0°—no disease symptoms, and 4°—over 50% of the root area infected) [75]. The disease index was calculated according to McKinney's formula [76]:

$$disease\ index = \frac{\sum(a_i \times b_i)}{n \times c} \times 100 \quad (1)$$

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 and c—highest score of the rating scale.

2.2. Laboratory Mycological Analysis

In each year of the study, the health of carrot plants was determined. According to the method described by Patkowska [23], 40 seedlings (BBCH 13-14) with disease symptoms were collected from particular experimental treatments for mycological analysis of the infected roots. Additionally, after the harvest (second decade of October), 40 randomly selected carrot roots (BBCH 49) from each experimental treatment with necrotic and etiological signs were subject to mycological analysis. The analysis was conducted according to the method described by Patkowska and Konopiński [77,78] for scorzonera and chicory roots and by Patkowska [23] for carrot. This analysis allowed one to determine the composition of fungi infecting carrot seedlings and roots.

According to the method described by Patkowska [23], the infected parts of plants were rinsed for 30 min under running tap water, subsequently they were disinfected in 1% sodium hypochlorite. Surface-disinfected plant material was rinsed three times in sterile distilled water. Three-millimeter fragments were cut from the thus prepared plant material and placed in 9-cm sterile Petri dishes on a solidified mineral medium with the following composition: 38 g saccharose, 0.7 g NH_4NO_3, 0.3 g KH_2PO_4, 0.3 g $MgSO_4 \times 7H_2O$, 20 g agar and trace quantities of $FeCl_3 \times 6\ H_2O$, $ZnSO_4 \times 7\ H_2O$, $CuSO_4 \times 7\ H_2O$ and $MnSO_4 \times 5\ H_2O$ [23]. In each of the experimental treatment, 100 fragments of infected 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 cycles of 12 h light/12 h darkness [23,79]. 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 available keys and monographs [80–95]. Additionally, the fungi of the genus *Fusarium* were identified on PDA and SNA (selective nutrient agar) medium [96]. Malt and Czapek-Dox media were used for *Penicillium* sp. [97]. The number and percentage of occurrence of the recovered fungal species were calculated.

2.3. Laboratory Analysis of Microbial Communities

Nine weeks after sowing carrot seeds, rhizosphere soil samples were collected from each experimental treatment and microbiological laboratory analysis was conducted according to the method described by Czaban et al. [98] for wheat and by Patkowska [13,23] for common bean and carrot; ten carrot plants were dug out as a whole from each plot (i.e., 40 plants from each combination). The soil directly adhering to the carrot roots (i.e., rhizosphere soil) was shaken off into sterile Petri dishes. Under sterile laboratory conditions, soil samples from the same experimental treatment were mixed, then weighed in 10 g quantities and prepared for further analyses (4 replicates for each experimental treatment).

According to the method described by Patkowska [13,23], soil solutions were prepared in laboratory conditions from 10 g weighed amounts with dilutions from 10^{-1} to 10^{-7}. The total size of bacterial population was determined on nutrient agar (in Petri dishes). Tryptic soy agar was used for bacteria from the genus *Bacillus*, whereas *Pseudomonas* agar F was used for *Pseudomonas* spp. For isolation of *Bacillus* spp., soil dilutions were heated for 20 min at 80 °C. Martin's medium was used to determine the number of fungi. After 2–7 days of incubation at 20–22 °C, the number of bacterial and fungal colonies was determined and converted into CFU/g of soil DW (colony forming units/g of soil dry weight) [13,23]. The obtained fungal colonies were transferred to sterile Petri dishes with PDA medium and incubated for the next 14–24 days. After that time, the fungi were microscopically determined to the genus and species, based on the available monographs [80–97]. The number of obtained species of fungi was calculated.

2.4. Antagonistic Activity of Selected Rhizosphere Fungi of Carrot

According to the method described by Patkowska et al. [99] and by Mańka and Mańka [100], the obtained rhizosphere isolates of *Albifimbria verrucaria*, *Clonostachys rosea*, *Penicillium aurantiogriseum*, *Penicillium glabrum*, *Talaromyces flavus* and *Trichoderma* sp. from individual experimental treatments were used to determine their antagonistic effect towards selected fungi pathogenic to carrot (*Alternaria dauci*, *Alternaria radicina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*).

The experiments were conducted on Petri dishes with sterile PDA medium. In the central part of the dish, two 3-mm fungi inocula were grafted 2 cm apart. Colonies of the studied fungi grown from one, 3-mm inoculum grafted in the middle of the dish served as controls. Cultures were grown in an incubator at a temperature of 24 °C. The biotic effect was established after 10 days of growth [101].

The phytopathological function is expressed as the individual biotic effect (IBE), i.e., the effect of one isolate of a given species on pathogens. IBE multiplied by species frequency gives the general biotic effect (GBE), considered as the effect of all isolates on the pathogen. The summary biotic effect (SBE) is obtained after adding all GBEs. The summary biotic effect of saprotrophic fungi on the studied pathogenic fungi from individual experimental treatments allowed us to determine their antagonistic activity in the carrot rhizosphere [99].

2.5. Statistical Analysis

Results concerning the emergence, health status, carrot disease index and the population of rhizosphere microorganisms were statistically analyzed. The significance of differences was determined on the basis of Tukey's confidence intervals ($p < 0.05$). Statistical calculations were carried out using Statistica, version 6.0 (StatSoft, Krakow, Poland).

3. Results

The experiments showed that plant density in the experimental plots grown from the seeds dressed with the fungicide Zaprawa Nasienna T 75 DS/WS was similar to the number of plants obtained after the application of biostimulants. The mean number of carrot seedlings in all experimental treatments ranged from 61.4 to 84.1 plants/m^2 (Table 1). The best emergence was observed after the application of Trianum P (84.1 seedlings, on average) or the fungicide (82.1). The number of seedlings grown on plots with Beta-Chikol or Timorex Gold 24 EC was smaller and amounted to 74.6 or 72.4, respectively. The worst emergence (61.4) was observed without any protective treatments. Nevertheless, seedlings with disease symptoms grew on each plot. After digging out the seedlings, brown necrotic spots were visible on the roots (Figure 1). Disease symptoms in the form of rot or dry necrosis with mycelium hyphae were also observed on the roots after carrot harvest (Figures 2 and 3). The average percentage of diseased seedlings ranged from 2.3% (Trianum P) to 11.3% (control). The application of Trianum P, Beta-Chikol and Timorex Gold 24 EC considerably reduced plant infection as the proportion of seedlings with disease symptoms was lower than in the control (2.3%, 3.6% and 7%, respectively; Table 1).

Table 1. Plant density and health status of carrot seedlings.

Experimental Treatment	Field Stand per 1 m^2				Diseased Seedlings (%)			
	2014	2015	2016	Mean	2014	2015	2016	Mean
Trianum P	84.8 a	91.4 a	76.2 a	84.1 a	1.5 c	2.5 c	3.0 c	2.3 c
Beta-Chikol	76.6 b	83.6 b	63.6	74.6 b	2.5 c	4.0 bc	4.5 c	3.6 c

Table 1. *Cont.*

Experimental Treatment	Field Stand per 1 m^2				Diseased Seedlings (%)			
	2014	**2015**	**2016**	**Mean**	**2014**	**2015**	**2016**	**Mean**
Timorex Gold 24 EC	75.0 b	81.4 b	61.0 b	72.4 b	5.0 b	7.5 b	8.5 b	7.0 b
Zaprawa Nasienna T 75 DS/WS	82.4 a	90.6 a	73.4 a	82.1 a	2.0 c	3.0 c	4.0 c	3.0 c
Control	62.2 c	70.0 c	52.2 c	61.4 c	9.5 a	11.5 a	13.0 a	11.3 a

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

(**a**) (**b**)

Figure 1. Carrot seedlings: (**a**) without necrosis on the roots and (**b**) infected by fungi (photo by E. Patkowska).

(**a**) (**b**)

Figure 2. Carrot roots: (**a**): *Sclerotinia sclerotiorum* on the carrot roots; (**b**) sclerotia of *Sclerotinia sclerotiorum* on the carrot roots; (photo by E. Patkowska).

(**a**) (**b**)

Figure 3. *Sclerotinia sclerotiorum*: (**a**) sclerotia and mycelium; (**b**) sclerotia; (photo by E. Patkowska).

The indicator of the protective effect of the applied biostimulants against carrot infection by plant pathogens was the value of the disease index of the seedlings. The disease index of carrot seedling roots was on average 14.5 for all experimental treatments (Figure 4). Trianum P and Zaprawa Nasienna T 75 DS/WS were the most effective in protecting the seedlings against fungal infection, because the disease index was the lowest (10.3 and 11.3, respectively). These values were significantly lower than in the control (21.2). Slightly higher values of the disease index were recorded after the application of Beta-Chikol (14.1) and Timorex Gold 24 EC (15.5). They were not significantly different, but also lower than in the control.

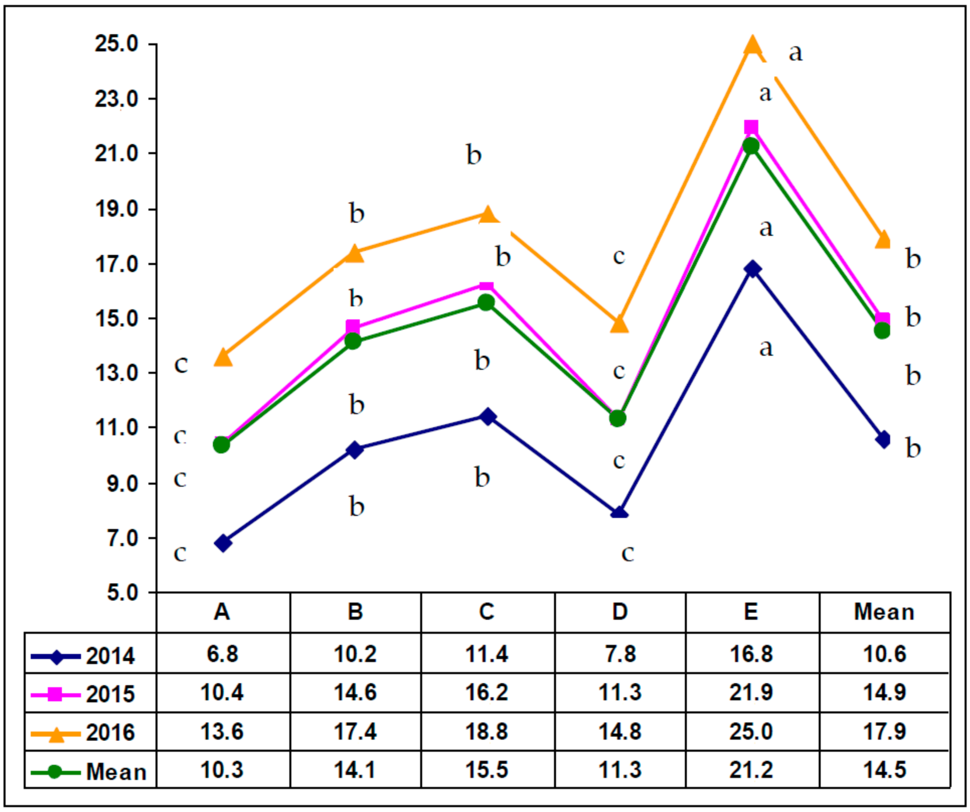


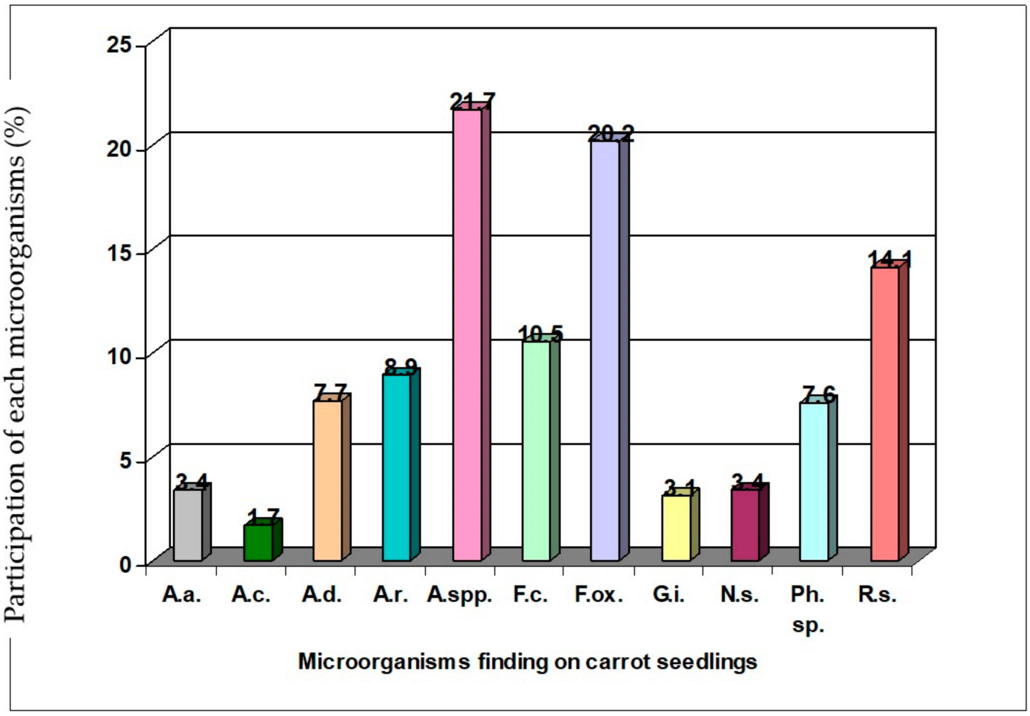
Figure 4. Values of the disease index of carrot seedlings. A—Trianum P, B—Beta-Chiko with l, C—Timorex Gold 24 EC, D—Zaprawa Nasienna T 75 DS/WS, E—control. Values for years marked the same letter do not differ significantly at $p \leq 0.05$.

Carrot plants were colonized by both pathogenic and saprotrophic fungi. A total of 1379 colonies of fungi and fungus-like organisms belonging to 11 genera were isolated from diseased carrot seedlings (Table 2). *Alternaria* sp., *Fusarium* sp. and *Rhizoctonia solani* fungi were clearly the dominant pathogens. The genus *Alternaria* was represented by the species *Alternaria alternata*, *A. consortialis*, *A. dauci* and *A. radicina* and their total proportion was 3.4%, 1.7%, 7.7% and 8.9%, respectively (in total—21.7%; Figure 5). The genus *Fusarium* was represented by the species *Fusarium culmorum* (10.5%) and *F. oxysporum* (20.2%). In addition, the following microorganisms, considered as potential pathogens, were isolated from the diseased carrot seedlings: *Rhizoctonia solani* (14.1%), *Phytophthora* sp. (7.6%), *Neocosmospora solani* (3.4%) and *Globisporangium irregulare* (3.1%). The smallest population of these microorganisms colonized the seedlings after Trianum P application. Slightly higher numbers were found in the plots with Beta-Chikol and Timorex Gold 24 EC, and the highest in control.

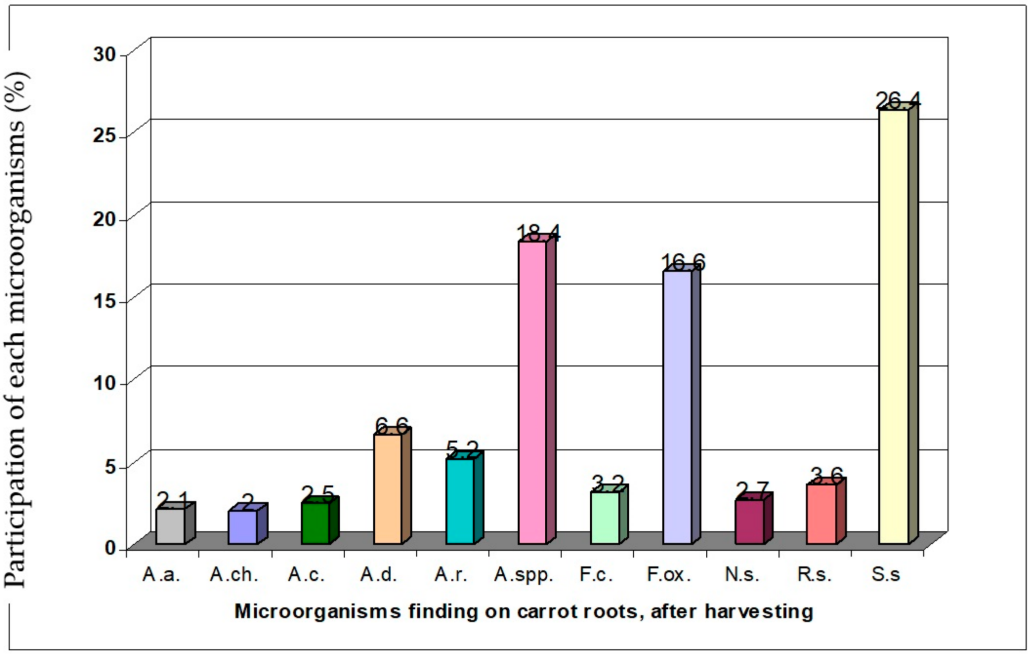
Table 2. Microorganisms isolated from diseased carrot seedlings (sum from 2014 to 2016).

Microorganisms	Experimental Treatment/Number of Isolates					
	A	**B**	**C**	**D**	**E**	**Total**
Alternaria alternata (Fr.) Keissler	5	9	11	7	15	47
Alternaria consortialis (Thüm.) J.W. Groves and S. Hughes	1	5	7	3	8	24
Alternaria dauci (Kühn) Groves and Skolko	11	20	26	15	34	106
Alternaria radicina Meier, Drechsler and Eddy	12	24	31	17	39	123
Clonostachys rosea (Link) Schroers, Samuels, Seifert	7	5	4	-	-	16
Cylindrocarpon didymum (Harting) Wollenw.	2	7	9	4	11	33
Epicoccum nigrum Link	6	15	19	10	25	75
Fusarium culmorum (W.G.Sm.) Sacc.	17	29	34	22	43	145
Fusarium oxysporum Schl.	34	54	63	42	85	278
Globisporangium irregulare (Buisman) Uzuhashi, Tojo and Kakish.	4	8	10	6	15	43
Neocosmospora solani (Mart.) L. Lombard and Crous	5	10	12	7	13	47
Penicillium lividum Westling	8	16	20	12	24	80
Penicillium thomii Marie	-	5	8	3	10	26
Phytophthora sp.	9	22	27	14	33	105
Rhizoctonia solani J.G. Kühn	22	38	46	30	58	194
Trichoderma sp.	17	11	6	3	-	37
Total isolates	160	278	333	195	413	1379

A—Trianum P, B—Beta-Chikol, C—Timorex Gold 24 EC, D—Zaprawa Nasienna T 75 DS/WS, E—control.



(**a**)



(**b**)

Figure 5. Total participation of selected microorganisms isolated from carrot plants in 2014–2016: (**a**) carrot seedlings and (**b**) carrot roots; A.a.—*Alternaria alternata*, A.ch.—*Alternaria chartarum*, A.c.—*Alternaria consortialis*, A.d.—*Alternaria dauci*; A.r.—*Alternaria radicina*, A.spp.—*Alternaria* spp., F.c.—*Fusarium culmorum*; Fox.—*Fusarium oxysporum*; G.i.—*Globisporangium irregulare*, N.s.—*Neocosmospora solani*, Ph.sp.—*Phytophthora* sp.; R.s.—*Rhizoctonia solani*, S.s.—*Sclerotinia sclerotiorum*.

After harvest, 1162 colonies of microorganisms belonging to 14 genera were obtained from carrot roots (Table 3). *Alternaria dauci* (6.6%), *A. radicina* (5.2%), *A. consortialis* (2.5%), *A. alternata*

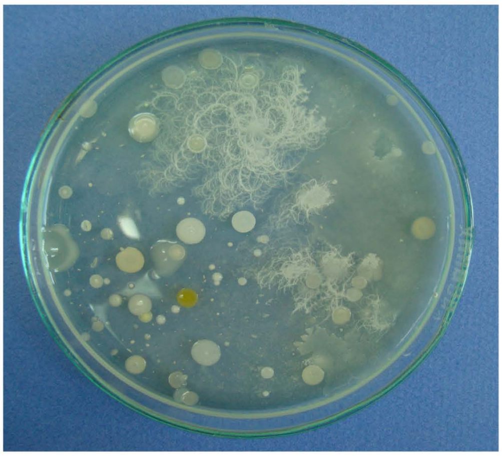
(2.1%), *A. chartarum* (2%), *Fusarium oxysporum* (16.6%), *F. culmorum* (3.2%), *Neocosmospora solani* (2.7%), *Rhizoctonia solani* (3.6%) and *Sclerotinia sclerotiorum* (26.4%) were also isolated from carrot roots after harvest (Figure 5). The greatest number of these fungi was obtained from control (without biostimulants and fungicide). *Trichoderma harzianum* T-22, chitosan and tea tree oil considerably reduced the occurrence of these microorganisms. Within saprotrophic fungi, *Clonostachys* sp., *Epicoccum* spp., *Gliomastix* sp., *Mucor* sp., *Penicillium* sp. and *Trichoderma* sp. were isolated from the diseased carrot seedlings and roots after harvest (Tables 2 and 3). Biostimulants, especially Trianum P and Beta-Chikol promoted their development.

Table 3. Microorganisms isolated from diseased carrot roots after harvest (sum from 2014 to 2016).

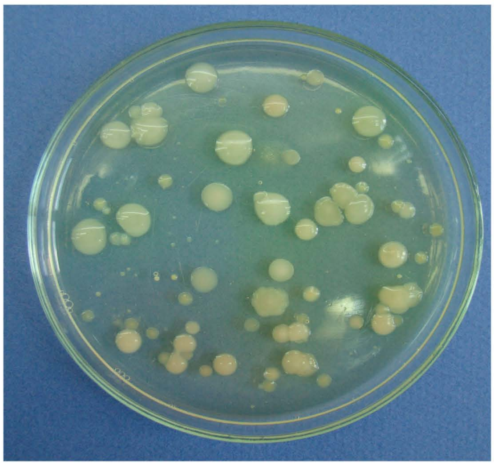
Microorganisms	**Experimental Treatment/Number of Isolates**					
	A	**B**	**C**	**D**	**E**	**Total**
Alternaria alternata (Fr.) Keissler	2	5	6	3	8	24
Alternaria chartarum Preuss	2	4	6	3	8	23
Alternaria consortialis (Thüm.) J.W. Groves and S. Hughes	4	6	6	5	8	29
Alternaria dauci (Kühn) Groves and Skolko	8	15	18	12	24	77
Alternaria radicina Meier, Drechsler and Eddy	7	12	14	9	18	60
Arthrinium phaeospermum (Corda) M.B. Ellis	-	-	2	-	5	7
Cylindrocarpon didymum (Harting) Wollenw.	-	5	7	-	12	24
Epicoccum nigrum Link	1	6	10	1	15	33
Fusarium avenaceum (Fr.) Sacc.	-	-	-	-	3	3
Fusarium culmorum (W.G.Sm.) Sacc.	3	7	9	5	13	37
Fusarium oxysporum Schl.	25	37	44	31	56	193
Gliomastix murorum (Corda) S. Hughes	-	-	6	-	11	17
Neocosmospora solani (Mart.) L. Lombard and Crous	1	5	9	1	15	31
Mucor plumbeus Bonord.	3	7	9	5	11	35
Penicillium dierckxii Biourge	9	14	19	11	25	78
Penicillium janczewskii Zalessky	7	14	17	10	23	71
Rhizopus stolonifer (Ehrenb.) Vuill.	-	3	5	-	12	20
Rhizoctonia solani J.G. Kühn	4	8	9	6	15	42
Sclerotinia sclerotiorum (Lib.) de Bary	40	60	69	49	89	307
Torula herbarum (Pers.) Link	-	-	1	-	3	4
Trichoderma sp.	20	14	11	2	-	47
Total isolates	136	222	277	153	374	1162

A—Trianum P, B—Beta-Chikol, C—Timorex Gold 24 EC, D—Zaprawa Nasienna T 75 DS/WS, E—control.

The number of colonies of carrot rhizosphere microorganisms isolated in vitro on selective media varied (Figure 6). The total population of bacteria ranged on average from 2.05×10^6 to 7.01×10^6 CFU/g of soil DW (Table 4). The smallest population of bacteria was found in the rhizosphere of control plants, while the biggest in the rhizosphere of carrots after the application of Zaprawa Nasienna T 75 DS/WS. The population of bacteria colonizing the rhizosphere of carrot plants treated with Trianum P was statistically higher (6.82×10^6 CFU/g of soil DW) than after Beta-Chikol application (4.51×10^6 CFU/g of soil DW) and Timorex Gold 24 EC (4.36×10^6 CFU/g of soil DW). A similar relationship was observed for the population of *Bacillus* sp. and *Pseudomonas* sp. Their population ranged from 0.99×10^6 to 4.81×10^6 CFU/g of soil DW and from 0.23×10^6 to 2.12×10^6 CFU/g of soil DW, respectively. Independently of the applied biostimulants and fungicide, *Bacillus* sp. was more abundant in carrot roots as compared to *Pseudomonas* sp. A reverse relationship was found for the fungal population, which ranged on average from 3.05×10^3 to 9.37×10^3 CFU/g of soil DW. The rhizosphere of the control plants was colonized by fungi to the highest degree. Each of the applied preparations limited the development of fungi in the rhizosphere of carrot. Their population was statistically smaller than in the control. Trianum P and Zaprawa Nasienna T 75 DS/WS proved to be particularly effective (Table 4).

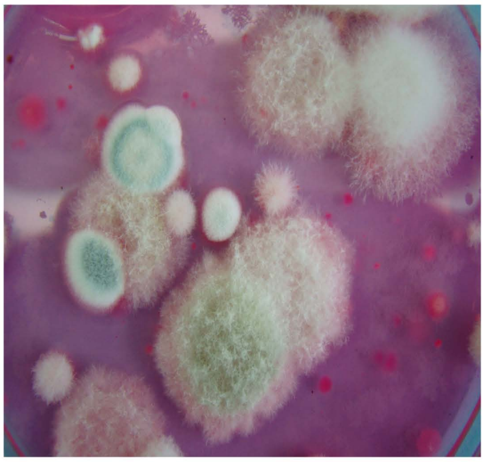


(**a**)



(**b**)

(**c**)



(**d**)

Figure 6. Microorganisms isolated from the rhizosphere of carrot in Petri dishes: (**a**,**b**) bacteria; (**c**,**d**) fungi (photo by E. Patkowska).

Table 4. Number of bacteria and fungi isolated from the rhizosphere of carrot in 2014–2016.

Experimental Treatment	Total CFU of Bacteria (10^6/g of Soil DW)				CFU of *Bacillus* sp. (10^6/g of Soil DW)				CFU of *Pseudomonas* sp. (10^6/g of Soil DW)				Total CFU of Fungi (10^3/g of Soil DW)			
	2014	**2015**	**2016**	**Mean**	**2014**	**2015**	**2016**	**Mean**	**2014**	**2015**	**2016**	**Mean**	**2014**	**2015**	**2016**	**Mean**
Trianum P	5.08 a	7.12 a	8.26 a	6.82 a	3.10 a	5.16 a	5.83 a	4.70 a	1.80 a	1.62 a	2.24 a	1.88 a	2.56 c	3.82 c	3.52 c	3.30 c
Beta-Chikol	3.14 b	4.25 b	6.15 b	4.51 b	2.13 b	3.20 b	4.14 b	3.16 b	1.00 a,b	1.02 a,b	1.92 a	1.31 a,b	4.98 b	5.98 b	6.15 b	5.70 b
Timorex Gold 24 EC	3.10 b	4.16 b	5.84 b	4.36 b	2.12 b	3.10 b	4.02 b	3.08 b	0.75 b	0.43 b	0.54 b	0.57 b	5.14 b	6.50 b	6.84 b	6.16 b
Zaprawa Nasienna T 75 DS/WS	5.24 a	7.34 a	8.45 a	7.01 a	3.14 a	5.28 a	6.00 a	4.81 a	2.05 a	2.00 a	2.31 a	2.12 a	2.32 c	3.68 c	3.14 c	3.05 c
Control	1.08 c	2.06 c	3.00 c	2.05 c	0.52 c	1.15 c	1.32 c	0.99 c	0.12 c	0.23 b	0.34 b	0.23 b	9.64 a	9.92 a	8.56 a	9.37 a

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

In total, 1394 isolates of fungi belonging to 17 genera were obtained from the carrot rhizosphere (Table 5). Their species composition was similar in all experimental treatments. On the other hand, the quantitative composition differed and depended on the applied preparation. The largest fungal population was isolated from the rhizosphere of carrot cultivated without any protective treatments (control)—408 isolates. Trianum P and Beta-Chikol considerably limited the growth of fungi, as 221 and 294 isolates, respectively, were obtained after their application. The effect of Timorex Gold 24 EC on the reduction of the fungal population was slightly smaller (309 isolates). The smallest fungal population was isolated from the rhizosphere of carrot protected with the fungicide Zaprawa Nasienna T 75 DS/WS (162 isolates). Fungi of the genera *Alternaria*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Rhizoctonia*, *Sclerotinia*, *Talaromyces* and *Trichoderma* were most frequently isolated. The dominant species *Fusarium oxysporum* (16.6%), *Rhizoctonia solani* (11.1%), *Alternaria alternata* (5%), *Sclerotinia sclerotiorum* (4.8%) and *Fusarium culmorum* (4.6%) (Figure 7) more often colonized the rhizosphere of carrot cultivated without any protective treatments than after the application of biostimulants (Table 5). *Trichoderma* sp., *Albifimbria verrucaria* and *Clonostachys rosea* dominated in the rhizosphere of saprotrophic fungi. They colonized carrot roots in experimental treatments with Trianum P, Beta-Chikol and Timorex Gold 24 EC more abundantly than with Zaprawa Nasienna T 75 DS/WS. Moreover, they were not isolated from the rhizosphere of control plants (Table 5).

Table 5. Fungi isolated from the rhizosphere of the carrot (sum from 2014 to 2016).

Fungus Species	**Experimental Treatment/Number of Isolates**					
	A	**B**	**C**	**D**	**E**	**Total**
Albifimbria verrucaria (Alb. and Schwein.) L. Lombard and Crous	20	19	12	-	-	51
Alternaria alternata (Fr.) Keissler	7	14	16	10	23	70
Alternaria chartarum Preuss	1	3	5	2	6	17
Alternaria dauci (Kühn) Groves and Skolko	-	1	4	-	8	13
Alternaria radicina Meier, Drechsler and Eddy	-	-	2	-	4	6
Aspergillus fumigatus Fresen.	4	7	8	6	12	37
Cladosporium herbarum (Pers.) Link	3	8	12	6	17	46
Clonostachys rosea (Link) Schroers, Samuels, Seifert	20	18	8	2	-	48
Epicoccum nigrum Link	1	7	11	2	18	39
Fusarium avenaceum (Fr.) Sacc.	1	7	10	3	18	39
Fusarium culmorum (W.G.Sm.) Sacc.	8	12	14	10	20	64
Fusarium oxysporum Schl.	30	41	51	36	74	232
Gliomastix murorum (Corda) S. Hughes	-	3	5	-	9	17
Mucor racemosus Fresenius	6	12	16	8	24	66
Neocosmospora solani (Mart.) L. Lombard and Crous	1	3	5	1	8	18
Penicillium aurantiogriseum Dierckx	8	6	3	5	10	32
Penicillium glabrum (Wehmer) Westling	9	4	2	7	15	37
Rhizoctonia solani J.G. Kühn	18	29	36	22	49	154
Rhizopus stolonifer (Ehrenb.) Vuill.	7	16	21	11	33	88
Sarocladium kiliense (Grütz) Summerb.	-	-	2	-	5	7
Sclerotinia sclerotiorum (Lib.) de Bary	9	13	15	11	19	67
Talaromyces flavus (Klöcker) Stolk and Samson	14	10	3	6	20	53
Talaromyces stipitatus (Thom ex C.W. Emmons) C.R. Benj.	4	10	14	7	16	51
Trichoderma sp.	50	51	34	7	-	142
Total isolates	221	294	309	162	408	1394

A—Trianum P, B—Beta-Chikol, C—Timorex Gold 24 EC, D—Zaprawa Nasienna T 75 DS/WS, E—control.

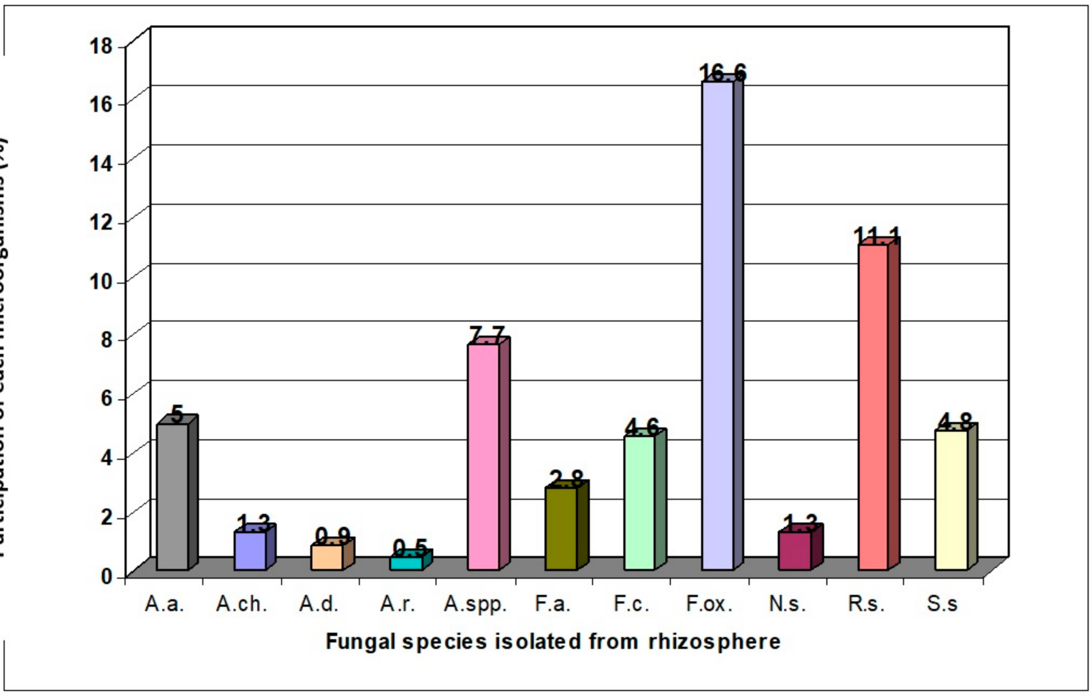


Figure 7. Total participation of selected fungi isolated from the rhizosphere of carrot in 2014–2016. A.a.—*Alternaria alternata*, A.c.—*Alternaria chartarum*, A.d.—*Alternaria dauci*, A.r.—*Alternaria radicina*, A.spp.—*Alternaria* spp., F.a.—*Fusarium avenaceum*, F.c.—*Fusarium culmorum*, F.ox.—*Fusarium oxysporum*, N.s.—*Neocosmospora solani*, R.s.—*Rhizoctonia solani*, S.s.—*Sclerotinia sclerotiorum.*

On the basis of laboratory tests, the number of antagonistic fungi (*Albifimbria verrucaria*, *Clonostachys rosea*, *Penicillium* spp., *Talaromyces flavus* and *Trichoderma* sp.) towards fungi pathogenic to the carrot (*Alternaria dauci*, *Alternaria radicina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*) was determined. Trianum P and Beta-Chikol were most effective in stimulating the development of antagonistic fungi (*Albifimbria verrucaria*, *Clonostachys rosea*, *Penicillium* spp., *Talaromyces flavus* and *Trichoderma* sp.) in the rhizosphere of carrots, because 121 and 108 isolates, respectively, were obtained after their application (Figure 8). A smaller population of antagonistic fungi was found when Timorex Gold 24 EC was used for carrot protection (62 isolates). The fungicide Zaprawa Nasienna T 75 DS/WS did not show such a positive effect (27 isolates). *Trichoderma* sp., *Clonostachys rosea* and *Albifimbria verrucaria* dominated among the antagonists.

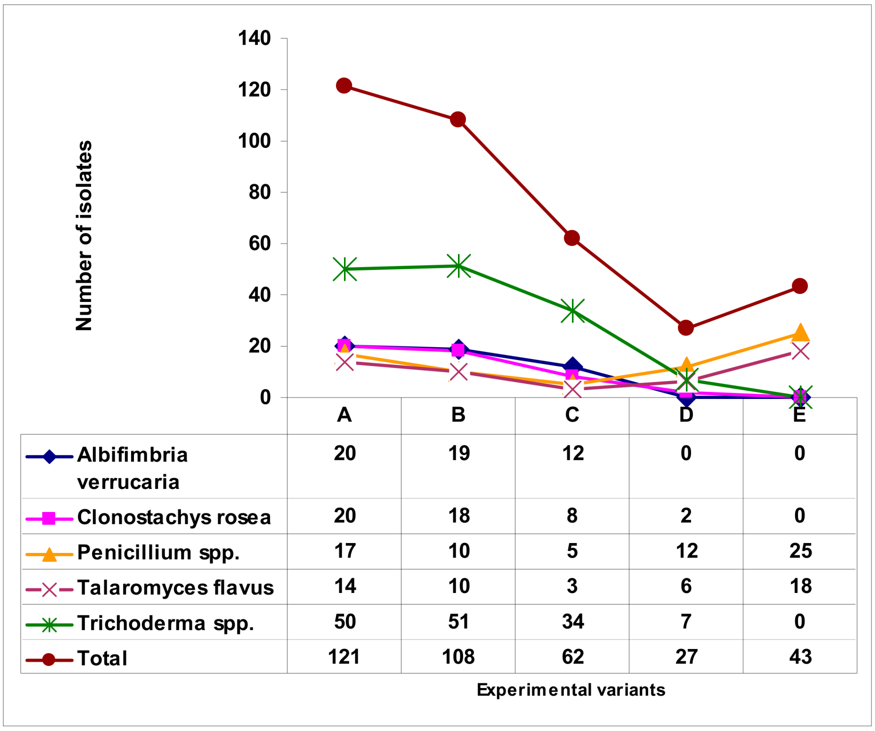


Figure 8. Number of antagonistic fungi isolated from the rhizosphere of carrot (sum from 2014–2016). A—Trianum P, B—Beta-Chikol, C—Timorex Gold 24 EC, D—Zaprawa Nasienna T 75 DS/WS, E—control.

The antagonistic activity of rhizosphere microorganisms depended on the applied preparation. It was the highest after Trianum P and Beta-Chikol application and slightly lower in combinations with Timorex Gold 24 EC and Zaprawa Nasienna T 75 DS/WS. The antagonistic activity of the tested fungi was the weakest in the control (without biostimulants and fungicide; Table 6).

Regardless of the applied preparation, antagonistic fungi were most effective in inhibiting the growth of *Alternaria radicina* and *A. dauci.* The summary biotic effect (SBE) towards those pathogens after Trianum P and Beta-Chikol application amounted to +721, +687 and +685, +657, respectively (Table 6). Timorex Gold 24 EC and Zaprawa Nasienna T 75 DS/WS stimulated antagonists in limiting the growth of *Alternaria radicina* and *A. dauci* to a lesser extent (SBE: +408, +397 and +111, +103, respectively). The antagonistic activity of saprotrophic fungi isolated from the carrot rhizosphere against *Rhizoctonia solani* and *Sclerotinia sclerotiorum* was slightly weaker. The summary biotic effect (SBE) against these two pathogens was +619 and +593 for Trianum P, +596 and +575 for Beta-Chikol, +366 and +354 for Timorex Gold 24 EC, +94 and +93 for Zaprawa Nasienna T 75 DS/WS and +79 and +71 for control, respectively (Table 6). The highest antagonistic activity among saprotrophic fungi was shown by *Trichoderma* sp. (Figure 9). Their individual biotic effect (IBE) in relation to all the tested pathogenic fungi was +8 (Table 6).

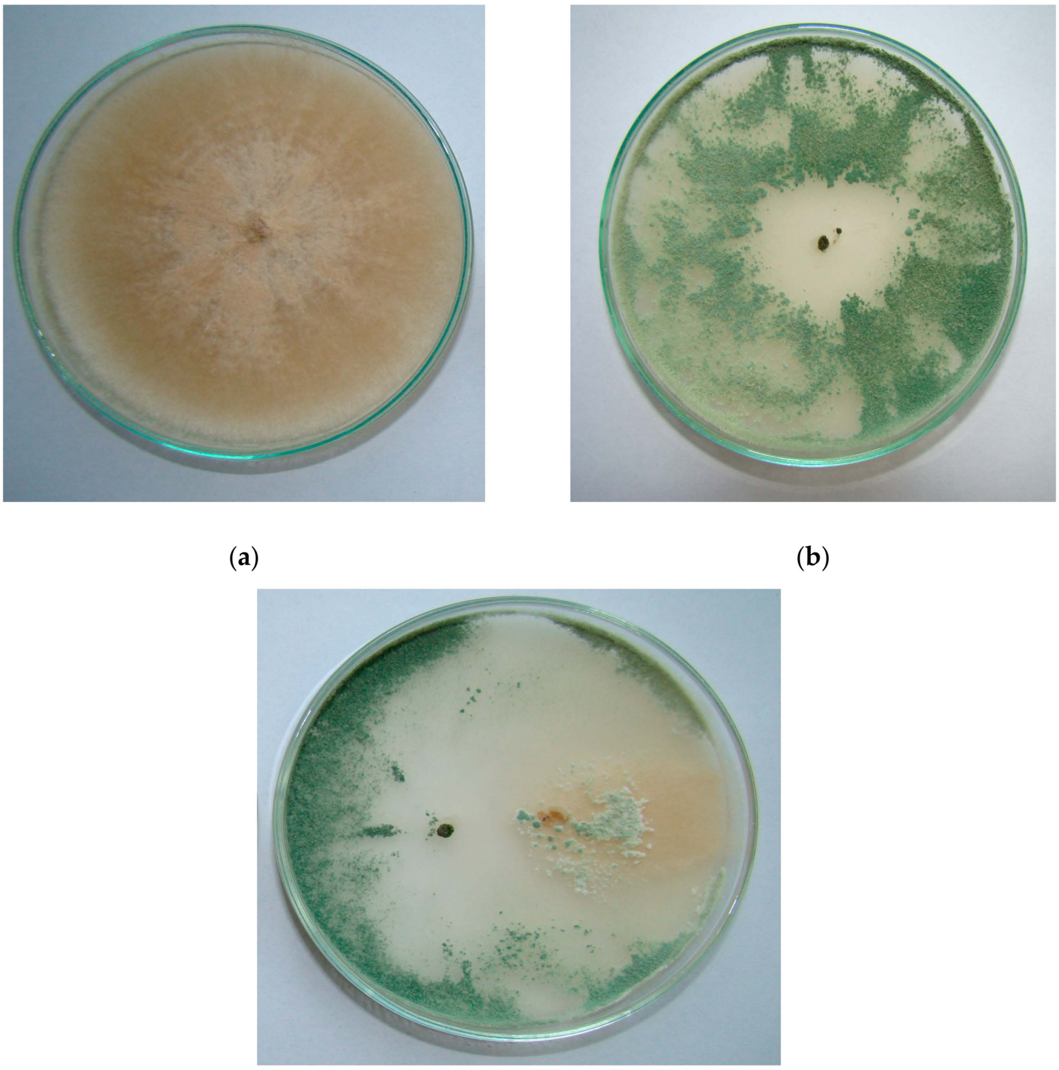
Table 6. Antagonistic activity of selected saprotrophic fungi isolated from the carrot rhizosphere towards pathogenic fungi.

Fungi	Number of Isolates	*Alternaria dauci*		*Alternaria radicina*		*Rhizoctonia solani*		*Sclerotinia sclerotiorum*	
		IBE*	GBE**	IBE*	GBE**	IBE*	GBE**	IBE*	GBE**
Trianum P									
Albifimbria verrucaria (Alb. and Schwein.) L. Lombard and Crous	20	+5	+100	+5	+100	+4	+80	+3	+60
Clonostachys rosea (Link) Schroers, Samuels, Seifert	20	+6	+120	+7	+140	+4	+80	+4	+80
Penicillium aurantiogriseum Dierckx	8	+2	+16	+2	+16	+1	+8	+2	+16
Penicillium glabrum (Wehmer) Westling	9	+1	+9	+1	+9	+1	+9	+1	+9
Talaromyces flavus (Klöcker) Stolk and Samson	14	+3	+42	+4	+56	+3	+42	+2	+28
Trichoderma sp.	50	+8	+400	+8	+400	+8	+400	+8	+400
Number of isolates	121								
SBE* **			**+687**		**+721**		**+619**		**+593**
Beta-Chikol									
Albifimbria verrucaria (Alb. and Schwein.) L. Lombard and Crous	19	+5	+95	+5	+95	+4	+76	+3	+57
Clonostachys rosea (Link) Schroers, Samuels, Seifert	18	+6	+108	+7	+126	+4	+72	+4	+74
Penicillium aurantiogriseum Dierckx	6	+2	+12	+2	+12	+1	+6	+2	+12
Penicillium glabrum (Wehmer) Westling	4	+1	+4	+1	+4	+1	+4	+1	+4
Talaromyces flavus (Klöcker) Stolk and Samson	10	+3	+30	+4	+40	+3	+30	+2	+20
Trichoderma sp.	51	+8	+408	+8	+408	+8	+408	+8	+408
Number of isolates	108								
SBE* **			**+657**		**+685**		**+596**		**+575**
Timorex Gold 24 EC									
Albifimbria verrucaria (Alb. and Schwein.) L. Lombard and Crous	12	+5	+60	+5	+60	+4	+48	+3	+36
Clonostachys rosea (Link) Schroers, Samuels, Seifert	8	+6	+48	+7	+56	+4	+32	+4	+32
Penicillium aurantiogriseum Dierckx	3	+2	+6	+2	+6	+1	+3	+2	+6
Penicillium glabrum (Wehmer) Westling	2	+1	+2	+1	+2	+1	+2	+1	+2
Talaromyces flavus (Klöcker) Stolk and Samson	3	+3	+9	+4	+12	+3	+9	+2	+6
Trichoderma sp.	34	+8	+272	+8	+272	+8	+272	+8	+272
Number of isolates	62								
SBE* **			**+397**		**+408**		**+366**		**+354**

Table 6. *Cont.*

Fungi	Number of Isolates	*Alternaria dauci*		*Alternaria radicina*		*Rhizoctonia solani*		*Sclerotinia sclerotiorum*	
		IBE*	**GBE****	**IBE***	**GBE****	**IBE***	**GBE****	**IBE***	**GBE****
Zaprawa Nasienna T 75 DS/WS									
Clonostachys rosea (Link) Schroers, Samuels, Seifert	2	+6	+12	+7	+14	+4	+8	+4	+8
Penicillium aurantiogriseum Dierckx	5	+2	+10	+2	+10	+1	+5	+2	+10
Penicillium glabrum (Wehmer) Westling	7	+1	+7	+1	+7	+1	+7	+1	+7
Talaromyces flavus (Klöcker) Stolk and Samson	6	+3	+18	+4	+24	+3	+18	+2	+12
Trichoderma sp.	7	+8	+56	+8	+56	+8	+56	+8	+56
Number of isolates	27								
SBE***			**+103**		**+111**		**+94**		**+93**
Control									
Penicillium aurantiogriseum Dierckx	10	+2	+20	+2	+20	+1	+10	+2	+20
Penicillium glabrum (Wehmer) Westling	15	+1	+15	+1	+15	+1	+15	+1	+15
Talaromyces flavus (Klöcker) Stolk and Samson	18	+3	+54	+4	+72	+3	+54	+2	+36
Number of isolates	43								
SBE***			**+89**		**+107**		**+79**		**+71**

IBE*—individual biotic effect; GBE**—general biotic effect; SBE***—summary biotic effect.



(**a**) (**b**) (**c**)

Figure 9. Ten-day-old colonies of fungi on the potato dextrose agar (PDA) medium: (**a**) *Rhizoctonia solani*; (**b**) *Trichoderma* sp. and (**c**) *Trichoderma* sp. and *Rhizoctonia solani* (photo by E. Patkowska).

4. Discussion

Trichoderma harzianum T-22 (Trianum P), chitosan (Beta-Chikol) and tea tree oil (Timorex Gold 24 EC) applied in this study promoted the growth and development of carrot plants and effectively protected them against infection by soil-borne pathogens. *T. harzianum* T-22, belonging to PGPMs, turned out to be more effective than chitosan and tea tree oil. Nevertheless, all biostimulants and the fungicide Zaprawa Nasienna T 75 DS/WS increased the emergence and plant density in the experimental plots. At the same time, after their application, a lower percentage of plants with disease symptoms was observed than in the control (without biostimulants and fungicide). The disease index of seedling roots after Trianum P and Zaprawa Nasienna T 75 DS/WS application was lower than after Beta-Chikol and Timorex Gold 24 EC application. The positive effect of chitosan on seed germination and emergence of soybean plants and the effect of grapefruit extract (Biosept 33 SL) on common bean and pea was demonstrated by Pastucha [102] and Pięta et al. [103], respectively.

In the current study, carrot seedlings and roots were colonized by saprotrophic and pathogenic fungi. Pathogenic fungi were represented by *Alternaria* spp., *Fusarium* spp., *Phytophthora* sp., *Neocosmospora solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. *Globisporangium irregulare* was also isolated from diseased seedlings. Biostimulants significantly reduced the colonization of the studied organs by these microorganisms. According to many authors [104–106], *Alternaria* spp. are common pathogenic species that cause diseases of various plants. Species of the genus *Alternaria*, such as *Alternaria radicina*, *A. dauci*, *A. petroselini* or *A. carotiincultae,* were reported on carrots in several countries [24,26,27,107,108]. Le Clerc et al. [33] and Ahmad and Siddiqui [109] found that *A. radicina*, *A. alternata* and *A. dauci* were highly harmful to carrot seedlings and roots. As reported by Kathe et al. [110], *A. radicina* is a fungal pathogen causing black rot disease of the carrot. According to Koutouan et al. [32], *Alternaria* leaf blight, caused by *A. dauci*, is the most damaging foliar disease affecting carrots. In a study by Szopińska et al. [1], carrot seedlings and seeds were infected mostly by fungi of the genera *Alternaria* and *Fusarium.* Moreover, Baturo-Cieśniewska et al. [35] and Siddiqui et al. [36] reported that carrot cultivation might be threatened by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

The tested *Trichoderma harzianum* T-22, chitosan and tea tree oil effectively limited the infestation of carrots by the above-mentioned fungi. At the same time, they favored the colonization of underground organs by saprotrophic fungi, especially *Clonostachys* sp., *Epicoccum* spp. and *Trichoderma* sp. A similar effectiveness of biological preparations and biostimulants (especially those based on *Trichoderma* and chitosan) was demonstrated by other authors in plant protection and growth promotion of various species [13,45,49,68,111–115]. The beneficial effect of chitosan on the emergence, health and yielding of plants from the family Fabaceae was confirmed by Patkowska [13] and Pięta et al. [103]. Biochikol 020 PC (a.s.—chitosan) effectively improved the health and yield of *Pisum sativum* [9]. Presowing treatment of pea seeds protected older plants against infection by *Fusarium culmorum*, *F. oxysporum*, *Alternaria alternata*, *Boeremia exigua*, *Haematonectria haematococca*, *Gibberella avenacea, Peyronellaea pinodes* and *Thanatephorus cucumeris* [9]. Chitosan was also used to dress the bulbs of ornamental plants [116] and in the protection of potato tubers against late blight and soft rot [117,118].

Laboratory and field studies have demonstrated the high efficiency of tea tree oil (Timorex Gold 24 EC) in limiting the abundance of *Bremia lactucae* on lettuce and high effectiveness in protecting this plant against downy mildew [119]. Single spraying of Timorex Gold effectively controlled and suppressed powdery mildew (*Sphaerotheca fuliginea*) on cucumber [15]. Moreover, tea tree oil showed high efficacy and strong healing effect against black Sigatoka in banana [39,40].

According to numerous authors [49,56,58,115], *Trichoderma* protects plants against soil and Phyllosphere pathogens. *Trichoderma harzianum* G 227 post-culture fluids, used for presowing seed dressing, had a positive effect on the number, health and yield of soybean plants [120]. They protected the germinating seeds, seedlings and older plants against infection by *Fusarium* spp., *Phoma exigua* var. *exigua*, *R. solani* and *S. sclerotiorum*. A study by Haikal [121] showed that *Trichoderma viride* spores and post-culture fluids reduced seed decay and soybean root rot caused by *Rhizoctonia solani*. Similarly, *T. harzianum* inhibited the development of soybean stem rot caused by *Sclerotinia sclerotiorum* [122]. As reported by Sánchez-Montesinos et al. [44], direct application of *Trichoderma aggressivum* f. *europaeum* to seeds in vitro did not increase the percentage of pepper and tomato seed germination, but demonstrated biostimulant properties under commercial plant nursery and greenhouse conditions.

Trichoderma harzianum T-22, chitosan, tea tree oil and the fungicide modified microorganism communities in the carrot rhizosphere. After their application, the size of rhizobacteria population, including *Bacillus* sp. and *Pseudomonas* sp., was greater than in the control. Each of the tested preparations, especially Trianum P and Zaprawa Nasienna T 75 DS/WS, reduced the size of rhizosphere fungal population. Biostimulants, especially Trianum P, reduced the occurrence of pathogenic fungi (*Fusarium oxysporum, F. culmorum, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Alternaria* spp.) and favored the development of antagonistic fungi (*Albifimbria* sp., *Clonostachys* sp., *Talaromyces* sp. and *Trichoderma* sp.) in the carrot rhizosphere. Many authors have shown great effectiveness of antagonistic fungi in inhibiting the development of pathogenic fungi and improving the health status of various plants [56,99,123,124]. As reported by Li et al. [125], the growth of soil-borne pathogens can be modified by root exudates. Earlier studies [41] showed the high efficacy of cover crops (buckwheat, white mustard and rye) in limiting the number of soil-borne fungi in carrot cultivation.

Moreover, chitosan used for the biological protection of runner bean [126], soybean [127] and common bean [13] significantly increased the population of *Bacillus* and *Pseudomonas* bacteria, while reducing the number of fungi in the rhizosphere of these plants. A similar relationship was observed with grapefruit extract [13,127] and Polyversum (containing *Pythium oligandrum* oospores) [13,127,128].

The effectiveness of tea tree oil in reducing the population of rhizosphere fungi in carrot cultivation could be due to its antiseptic properties. It is used in the control of phytopathogenic fungi and bacteria [15,72,119,129,130]. As reported by Li et al. [131] and Riccioni et al. [132], tea tree oil controlled a wide spectrum of pathogens of various plants (vegetables, field crops, herbs, fruit trees and grapevines), without causing any phytotoxic effects. Additionally, Carson et al. [69] reported antibacterial and anti-inflammatory properties of tea tree oil used in medicine [69].

Biostimulants used in the present study for the biological protection of carrots had a positive effect on the antagonistic activity of saprotrophic rhizosphere fungi towards the studied polyphages (*Alternaria dauci, Alternaria radicina, Rhizoctonia solani* and *Sclerotinia sclerotiorum*). *Trichoderma harzianum* T-22, as plant growth-promoting fungus, also showed great effectiveness, increasing the degree of colonization of carrot roots and rhizosphere soil by other antagonistic fungi. Such action significantly improved the health of the tested plant species. Błaszczyk et al. [57] reported that *Trichoderma* spp. positively affected plants by stimulating their growth and protecting against bacterial and fungal pathogens. Mycoparasitism [54,133–135], antibiosis [136–140] and competition [141–143] are the biocontrol mechanism by which *Trichoderma* spp. respond to the presence of phytopathogens, thereby preventing or impeding their development. These processes are stimulated by the biosynthesis of target metabolites, such as plant growth regulators, antibiotics, siderophores and lytic enzymes (especially chitinases, β-1,3-glucanases, β-1,6-glucanases), which completely degrade the cell walls of hyphae and spores [133,144–146]. The antagonistic activity of *Trichoderma* spp. against various pathogenic fungi has been described by many authors [56–58,63,124,133,147–150]. Błaszczyk et al. [151] reported the high activity of various Trichoderma strains towards toxigenic species such as *Fusarium avenaceum*, *F. cerealis*, *F. culmorum*, *F. graminearum* and *F. temperatum*. Strains of the species *Trichoderma longibrachiatum*, *T. atroviride* and *T. harzianum*, including *T. harzianum* T-22, showed the ability to reduce the synthesis of *Fusarium* mycotoxins [60,152]. The antagonistic activity of *T. harzianum* T-22 was confirmed, among others, against *Alternaria alternata* [153], *Sclerotinia sclerotiorum* [154,155] and *Rhizoctonia solani* [156].

5. Conclusions

In summary, the application of *Trichoderma harzianum* T-22 (Trianum P), chitosan (Beta-Chikol) and tea tree oil (Timorex Gold 24 EC) in carrot cultivation considerably improved the growth, development and health of this vegetable plant. They protected the germinating seeds and older plants from infection by soil-borne fungi. Their effect matched or exceeded the effect of the chemical substance tiuram (fungicide Zaprawa Nasienna T 75 DS/WS). Moreover, they had a positive influence on microbial communities in the rhizosphere. They reduced the population of pathogenic fungi colonizing carrot roots, while increasing the population of antagonistic fungi. The application of these preparations had a positive effect on the antagonistic activity of saprotrophic fungi against the studied polyphages (*Alternaria dauci*, *Alternaria radicina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*). On the basis of the present study and studies of other authors, these preparations can be regarded as factors improving the phytosanitary condition of the soil, which is of great importance in plant protection. Consequently, Trianum P, Beta-Chikol and Timorex Gold 24 EC can be recommended as biostimulants in ecological agricultural production, including *Daucus carota* cultivation.

Author Contributions: Conceptualization: E.P., M.B.-W., E.M. and A.J.; Methodology: E.P., E.M., A.J. and M.B.-W.; Data curation: E.P.; Formal analysis and software: E.P.; Validation: E.P., E.M., A.J., B.S.-B., M.B.-W.;

Writing—original draft preparation: E.P. and B.S.-B., Writing—review and editing: E.P., E.M., A.J., B.S.-B. and M.B.-W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed by the University of Life Sciences, project OKF/DS/2.

Acknowledgments: The authors thank reviewers for the valuable comments that have enriched the content of the article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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