# BIOLOGICAL CONTROL OF RUNNER BEAN (*PHASEOLUS COCCINEUS* L.) AGAINST SOIL-BORNE PATHOGENIC FUNGI

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## 1. Introduction

Biological control of different plant species against pathogenic factors consists of, for example, replacing pesticides with biopreparations based on antagonistic microorganisms, plant extracts or organic compounds [1, 3, 4, 8 - 11, 13 - 14]. The group of biopreparations that recently attracted a lot of attention includes Biochikol 020 PC, Biosept 33 SL and Polyversum.

Biosept 33 SL, containing 33% grapefruit extract, Polyversum, based on *Pythium oligandrum* oospores, and Biochikol 020 PC, whose active substance is chitosan, act directly on pathogenic factors and they induce plants' resistance to some pathogens [1, 4, 8 - 11, 13, 16, 20]. Studies by Orlikowski [8] on the mechanism of the effect of grapefruit extract on *Phytophthora cryptogea* showed that it limited the mycelium growth, inhibited the creation of zoosporangia and the germination of zoospores of this pathogen. Besides, grapefruit extract inhibited the growth of mycelium and limited the formation of conidial sporules and chlamydospores of *F. oxysporum* f. sp. *dianthi*, by which it considerably decreased the number of propagation units of this fungi in the subsoil [8]. According to Orlikowski and Jaworska-Marosz [9], at least 3 modes of *P. oligandrum* action on plant pathogens are known: mycoparasitisms mediated by intimate hypahae interactions, antibiosis, enhancement of plant. On the other hand, chitosan as resistance elicitor – through its contact with the plant – enhances the activity of genes, which set in motion the formation of biochemical compounds with fungistatic and fungicidal effects [16].

The purpose of the present studies was to establish the effect of the enumerated biopreparations on healthiness and yielding of *Phaseolus coccineus* and on the formation of microorganism communities in the rhizosphere of this plant, growing in the conditions of threat from soil-borne pathogenic fungi such as A*ltenaria alternata, Fusarium culmorum, F. oxysporum, F. solani, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

## 2. Material and methods

Studies were conducted in the years 2005-2006 on monoculture fields of runner bean with naturally accumulated infection material in the soil. Earlier studies [12, 15] pointed to the presence in this soil environment of such fungi pathogenic to this plant as *Alternaria alternata, Fusarium culmorum, F. oxysporum, F. solani, Pythium irregulare, Rhizoctonia solani, Sclerotinia sclerotiorum* (unpublished results of pathogenicity tests). The experiment was set according to the method described by Patkowska [13]. Before the sowing the seeds of runner bean 'Westa' cv. were dressed with 2.5% Biochikol 020 PC, 0.2% Biosept 33 SL, Polyversum (in the quantity 1 g × $100^{-1}$ g seeds) or Zaprawa Oxafun T (in the quantity 2 g × $kg^{-1}$ seeds). A control combination was also considered, i.e. without any seed dressing. The second protective treatment, consisting of spraying the plants with biopreparations or Bravo Plus 500 S.C. was performed at the beginning of runner bean anthesis.

The experiment established the population and healthiness of the seedlings and plants of runner bean at anthesis, whereas plants with disease symptoms were subjected to laboratory mycological analysis [5]. After the harvest the size of the seed yield and the proportion of seeds with necrotic spots on the seed cover were determined. Besides, a microbiological analysis of the rhizosphere soil was made at anthesis according to the method described by Patkowska [12] with the aim of determining the quantitative and qualitative composition of microorganisms occurring in particular experimental combinations. The results were statistically analyzed and the significance of differences was established on the basis of Tukey's confidence intervals [7].

## 3. Results and discussion

The applied biopreparations considerably improved the emergences, healthiness and yielding of runner bean. A greater number of plants were observed in the combinations with biopreparations as compared with the control. The best emergences were obtained on the plots sown with the seeds of bean dressed with Polyversum or Biosept 33 SL (94 seedlings, on average each). A slightly lower number of plants grew from the seeds dressed with Zaprawa Oxafun T (90 seedlings) or Biochikol 020 PC (on average, 86.5 seedlings), while the smallest number – in the control combination (70.5 seedlings, on average) (Table 1). Besides, Biosept 33 SL effectively protected the germinating seeds, and then the roots and the stem base, from infection by soil-borne fungi, since the proportion of infected seedlings in this combination was the smallest and it was 0.5%. A good protective effect was also shown by Polyversum, Biochikol 020 PC and Zaprawa Oxafun T (the proportion of infected seedlings was 1.2%, 1.3% and 1.3%, respectively). The greatest number of infected seedlings grew from the seeds that had not been dressed (3.7%) (Table 1).

A similar relation was found out in the second observation, i.e. at anthesis of runner bean. Then a slight loss of plants on particular plants as well as an increase of the number of infected plants was observed. The smallest proportion of infected plants at anthesis took place in the combination with Biosept 33 SL (0.8%), while the highest – in the control combination (4.9%) (Table 1).

Results obtained in the presented studies confirmed the information about the effectiveness of biopreparations in the protection of different plants from plant pathogens [1, 8-9, 11, 14, 17-18, 20].

After picking up plants from the plots of particular experimental combinations the studies determined the size and quality of the yield of runner bean seeds (Table 2). The size of the collected seed yield ranged from 1920 g to 3857 g from a plot. The greatest yield was collected from runner bean plants after the application of Biosept 33 SL or Biochikol 020 PC (respectively, 3857 g and 3564 g from a plot). Similar yielding was found for plants in the combinations with Polyversum and Zaprawa Oxafun T (respectively, 3080 g and 3070 g from a plot). The lowest yield (on average, 1920 g from a plot) with the highest proportion of infected seeds (6.8%, on average) was gathered from control plants. The smallest number of seeds with necrotic spots on the seed cover was found after the application of Biosept 33 SL (on average, 2.4%), and only slightly more in the combination with Biochikol 020 PC (3.7%, on average) or Polyversum (mean 3.6%) (Table 2).

As a result of mycological analysis of the infected roots and stem base of the seedlings and older plants of runner bean, 1923 fungi isolates were isolated from all experimental combinations (Table 3). The most frequently isolated fungi were those from genus *Fusarium* represented by *F. culmorum, F. oxysporum* f. sp. *phaseoli*, *F. solani* and *F. sporotrichioides*. *F. oxysporum* f. sp. *phaseoli* turned out to be the dominating one. The total proportion of this species isolated from runner bean seedlings constituted 25.4% while the proportion of plant at anthesis was 20.8% of all isolations. Besides, *Alternaria alternata, Phoma exigua, Rhizoctonia solani, Pythium irregulare* (only from the seedlings) as well as *Sclerotinia sclerotiorum* (only from plants at anthesis) were often isolated from infected plants. The effect of these fungi in infecting runner bean plants treated with biopreparations was smaller than in the control. Besides, species from genera *Acremonium, Cladosporium, Mucor, Rhizopus* and *Gliocladium, Penicillium* and *Trichoderma* were isolated from runner bean plants (Table 3).

***Table 1.*** *The number and healthiness of runner bean plants (mean from the years 2005-2006); * mean values in columns marked with the same letter do not differ significantly at $p \leq 0.05$.*

| Treatment | Concentration, % | Seedlings | | Plants at anthesis | |
|---|---|---|---|---|---|
| | | Number of seedlings on a plot | Participation of diseased seedlings on a plot, % | Number of plants on a plot | Participation of diseased plants on a plot, % |
| Polyversum | 0.1 | $94^{b}$ | $1.2^{b}$ | $92^{c}$ | $1.4^{ab}$ |
| Biochikol 020 PC | 2.5 | $86^{b}$ | $1.3^{b}$ | $85^{b}$ | $1.6^{b}$ |
| Biosept 33 SL | 0.2 | $94^{b}$ | $0.5^{a}$ | $92^{c}$ | $0.8^{a}$ |
| Zaprawa Oxafun T | 0.02 | $90^{b}$ | $1.3^{b}$ | $86^{b}$ | $1.9^{b}$ |
| Control | - | $70^{a}$ | $3.7^{c}$ | $66^{a}$ | $4.9^{c}$ |

***Table 2.*** *Yield and healthiness of runner bean seeds (mean from the years 2005-2006); * mean values in columns marked with the same letter do not differ significantly at $p \leq 0.05$.*

| Treatment | Concentration, % | Yield of runner bean seeds in g from the plot | Percentage of infected seeds |
|---|---|---|---|
| Polyversum | 0.1 | $3080^{b}$ | $3.6^{ab}$ |
| Biochikol 020 PC | 2.5 | $3564^{c}$ | $3.7^{b}$ |
| Biosept 33 SL | 0.2 | $3857^{d}$ | $2.4^{a}$ |
| Zaprawa Oxafun T | 0.02 | $3070^{b}$ | $3.9^{b}$ |
| Control | - | $1920^{a}$ | $6.8^{c}$ |

***Table 3.*** *Fungi isolated from infected of runner bean plants (sums of isolates from the years 2005-2006): Pol. – Polyversum, Bioch. – Biochikol 020 PC, Bios. – Biosept 33 SL, Z. Ox. – Zaprawa Oxafun T, C. - Control.*

| Fungus species | Treatment / Number of isolates | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Seedlings | | | | | | Plants at anthesis | | | | | | Total |
| | Pol. | Bioch | Bios. | Z. Ox. | C. | Mean | Pol. | Bioch | Bios. | Z. Ox. | C. | Mean | |
| *Acremonium murorum* (Corda) W. Gams | 1 | 4 | 3 | 7 | 2 | 17 | - | - | - | - | - | - | 17 |
| *Acremonium roseum* (Oud.) W. Gams | - | - | - | - | - | - | - | 2 | 3 | 3 | - | 8 | 8 |
| *Alternaria alternata* (Fr.) Keissler | 6 | 12 | 9 | 24 | 25 | 76 | 9 | 8 | 8 | 11 | 11 | 47 | 123 |
| *Aspergillus flavus* Link | - | - | - | - | - | - | 2 | 5 | 2 | 10 | 8 | 27 | 27 |
| *Aspergillus fumigatus* Fresenius | 2 | 2 | 1 | 7 | - | 12 | - | - | - | - | - | - | 12 |
| *Aureobasidium pullulans* (de Bary) Arnaud. | - | - | - | - | - | - | 2 | - | 1 | - | 1 | 4 | 4 |
| *Botrytis cinerea* Pers. | - | - | - | 2 | 1 | 3 | 4 | 4 | 5 | 11 | 13 | 37 | 40 |
| *Cladosporium cladosporioides* (Fres.) de Vries | 7 | 6 | 8 | 6 | 11 | 38 | 9 | 8 | 11 | 11 | 15 | 54 | 92 |
| *Cylindrocarpon decumbens* Wollenw. | 1 | - | - | 2 | 3 | 6 | 2 | - | - | 5 | 4 | 11 | 17 |
| *Epicoccum purpurascens* Ehr. ex. S. F. Gray | 2 | 3 | - | 1 | 3 | 9 | - | - | - | - | - | - | 9 |
| *Fusarium culmorum* (W. G. Sm.) Sacc. | 5 | 4 | 6 | 11 | 13 | 39 | 6 | 7 | 6 | 16 | 12 | 47 | 86 |
| *Fusarium oxysporum* Schl. f. sp. phaseoli Kend. Snyd. | 32 | 35 | 31 | 56 | 72 | 226 | 29 | 34 | 35 | 51 | 66 | 215 | 441 |
| *Fusarium solani* (Mart.) Sacc. | 10 | 9 | 9 | 17 | 18 | 63 | 11 | 9 | 9 | 21 | 23 | 73 | 136 |
| *Fusarium sporotrichioides* Sherb. | - | - | - | - | - | - | 2 | 2 | 2 | 3 | 5 | 14 | 14 |
| *Gliocladium catenulatum* Gilman Abbott | 11 | 21 | 13 | 3 | 3 | 51 | 9 | 18 | 11 | 4 | 3 | 45 | 96 |
| *Gliocladium fimbriatum* Gilman Abbott | 3 | 8 | 4 | - | - | 15 | 2 | 3 | 2 | - | 1 | 8 | 23 |
| *Gliocladium roseum* Bainier | - | - | - | - | - | - | 3 | 4 | 3 | - | - | 10 | 10 |
| *Humicola grisea* Traaen | 3 | - | 5 | 6 | 1 | 15 | 3 | 3 | - | 1 | 5 | 12 | 27 |
| *Mucor hiemalis* Wehmer | 2 | 2 | 3 | - | - | 7 | 8 | 7 | 10 | 5 | 8 | 38 | 45 |

***Table 3.a.*** *Fungi isolated from infected of runner bean plants (sums of isolates from the years 2005-2006): Pol. – Polyversum, Bioch. – Biochikol 020 PC, Bios. – Biosept 33 SL, Z. Ox. – Zaprawa Oxafun T, C. - Control.*

| Fungus species | Treatment / Number of isolates | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Seedlings | | | | | | Plants at anthesis | | | | | | Total |
| | Pol. | Bioch | Bios. | Z. Ox. | C. | Mean | Pol. | Bioch | Bios. | Z. Ox. | C. | Mean | |
| *Mucor mucedo* Fresenius | - | 2 | 1 | - | - | 3 | 3 | 6 | - | 4 | 3 | 16 | 19 |
| *Papulaspora irregularis* Hotsʹon | - | - | - | - | - | - | - | - | 2 | - | - | 2 | 2 |
| *Penicillium expansum* Link ex. S. F. Gray | - | - | 4 | - | 10 | 14 | 7 | 3 | 5 | 5 | 11 | 31 | 45 |
| *Penicillium notatum* Westling | 4 | 7 | 6 | 8 | 17 | 42 | - | - | - | - | - | - | 42 |
| *Penicillium purpurogenum* Stoll. | - | - | - | - | - | - | 6 | 5 | 3 | 12 | 11 | 37 | 37 |
| *Penicillium verrucosum* Dierckx var. *cyclopium* (West.) Samson et. al. | 4 | - | 4 | 10 | 19 | 37 | 4 | 7 | 10 | 5 | 11 | 37 | 74 |
| *Phoma eupyrena* Sacc. | - | - | - | - | - | - | 3 | - | 2 | 3 | 3 | 11 | 11 |
| *Phoma exigua* Desm. | 1 | 2 | 1 | 5 | 2 | 11 | 7 | 6 | 1 | 15 | 16 | 45 | 56 |
| *Pythium irregulare* Buisman | - | 3 | 1 | 14 | 13 | 31 | - | - | - | - | - | - | 31 |
| *Rhizoctonia solani* Kűhn | 7 | 5 | 3 | 20 | 20 | 55 | 3 | 4 | 7 | 7 | 9 | 30 | 85 |
| *Rhizopus nigricans* Ehrenberg | - | - | - | - | - | - | 8 | 5 | 1 | 6 | 9 | 29 | 29 |
| *Sclerotinia sclerotiorum* (Lib.) de Bary | - | - | - | - | - | - | 4 | 5 | 3 | 11 | 12 | 35 | 35 |
| Talaromyces flavus (Kocker) Stolk et Samson | - | 4 | 4 | 5 | 4 | 17 | - | - | - | - | - | - | 17 |
| *Torula herbarm* Link | - | - | - | - | - | - | - | 3 | - | 2 | - | 5 | 5 |
| *Trichoderma aureoviride* Rifai | 5 | 6 | 5 | 2 | 2 | 20 | 3 | 4 | 2 | 1 | - | 10 | 30 |
| *Trichoderma harzianum* Rifai | - | - | - | - | - | - | 8 | 12 | 9 | 4 | 3 | 36 | 36 |
| *Trichoderma koningii* Oud. | 14 | 24 | 18 | 4 | 4 | 64 | 11 | 17 | 11 | 1 | 2 | 42 | 106 |
| *Trichoderma viride* Pers. ex. S.F. Gray | 6 | 4 | 6 | 1 | - | 17 | - | 11 | 4 | 2 | 2 | 19 | 36 |
| **Total** | **126** | **163** | **145** | **211** | **243** | **888** | **168** | **202** | **168** | **230** | **267** | **1035** | **1923** |

It can be supposed that the proportion of such fungi as *A. alternate, Fusarium* spp., *P. irregulare, R. solani* and *S. sclerotiorum* isolated from the roots and the stem base of the examined plants was much smaller in the combinations with biopreparations as compared with the control, which was the result of direct effect of active substances contained in these preparations on plant pathogens. According to Orlikowski and Skrzypczak [11], 7-geranoxicumarin, found in grapefruit extract, can have inhibiting effect on pathogenic fungi and may induce resistance of gerbera to *P. cryptogea* [8]. A study by Orlikowski and Skrzypczak [10] showed that grapefruit extract has a wide range of activity. Applied as soil drench it strongly decreased population density of *F. oxysporum* f. sp. *dianthi* and *Pythium ultimum*. The application of grapefruit extract as a plant spray inhibited the development of willow rust, *Myrothecium* leaf spot of diffenbachia and grey mould of tulip [10]. On the other hand, the protective effect of Polyversum is linked to the mode of action of mycoparasite *P. oligandrum* on pathogens, which consists in antibiosis or parasitism. A study by Orlikowski and Jaworska-Marosz [9] showed that mycoparasite *P. oligandrum* may decrease population density of *F. oxysporum* f. sp. *dianthi* and suppressed the spread of *Fusarium* wilt of carnation. *In vitro* study showed an intimate contact of *F. oxysporum* f. sp. *dianthi* and *P. oligandrum* hyphae [9].

As a result of mycological analysis of runner bean seeds, 670 fungi isolates belonging to 16 species were obtained from all experimental combinations (Table 4). The fewest fungi were obtained from the seeds after applying Biosept 33 SL or Biochikol 020 PC, slightly more after using Polyversum, and the most in the control combination. The dominating were *F. oxysporum, A. alternata, B. cinerea, R. solani* and *S. sclerotiorum. Fusarium* spp. (*F. equiseti, F. oxysporum, F. poae,* and *F. sporotrichioides*) constituted the highest proportion. Besides, fungi from genera *Acremonium, Cladosporium, Epicoccum, Penicillium* and *Rhizopus*, among others, were isolated from the studied seeds (Table 4).

The microbiological analysis of the rhizosphere soil showed that totally the most bacteria occurred after applying Polyversum (mean $4.86x10^6$ cfu.) and Biosept 33 SL (mean $5.74\times10^6$ cfu.), slightly less in the combination with Biochikol 020 PC (mean $4.5\times10^6$), and the least in the control combination (Table 5). The population of bacteria from genus *Pseudomonas* was smaller than the population of bacteria from genus *Bacillus*. The most *Bacillus* spp. occurred in the rhizosphere of runner bean after the application of Polyversum (mean $2.67\times10^6$ cfu.), while the most *Pseudomonas* appeared in the combination with Biosept 33 SL and Biochikol 020 PC. On the other hand, the smallest population of fungi totally was observed in the rhizosphere after using Biosept 33 SL (mean $11.29\times10^3$ cfu.) and Biochikol 020 PC (mean $15.28\times10^3$ cfu.), slightly bigger after introducing Polyversum or Zaprawa Oxafun T into the soil, and the biggest in the control combination (mean $26.25\times10^3$ cfu.) (Table 5). It should be supposed that the biopreparations introduced into the soil had a positive effect on the composition of microorganism communities in the rhizosphere of the studied plant, since – as reported by Myśków [6] – proper proportions take place between the populations of microorganisms in the soil. When bacteria occur in a big population, the development of fungi is weakened, and *vice versa*.

***Table 4.*** *Fungi isolated from seeds of runner bean (sums of isolates from the years 2005-2006); 1 - seeds with spots, 2 - seeds without spots.*

| Fungal species | Treatment / Number of isolates | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Polyversum | | Biochikol 020 PC | | Biosept 33 SL | | Zaprawa Oxafun T | | Control | | Mean | Mean | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | |
| *Acremonium murorum* (Corda) W. Gams | 2 | - | 1 | - | 1 | - | 4 | 1 | 1 | - | 9 | 1 | 10 |
| *Alternaria alternata* (Fr.) Keissler | 7 | 2 | 4 | 1 | 3 | 1 | 6 | 2 | 9 | 5 | 29 | 11 | 40 |
| *Aspergillus niger* van Tiegh | 1 | - | 2 | - | 2 | - | 6 | 1 | 7 | 1 | 18 | 2 | 20 |
| *Botrytis cinerea* Pers. | 13 | 3 | 8 | 3 | 9 | 4 | 13 | 4 | 13 | 7 | 56 | 21 | 77 |
| *Cladosporium cladosporioides* (Fres) de Vries | 7 | 1 | 9 | 2 | 5 | 4 | 5 | - | 9 | 3 | 35 | 10 | 45 |
| *Epicoccum purpurascens* Ehr. ex. Schl. | 1 | - | 2 | - | - | - | 3 | - | 6 | 1 | 12 | 1 | 13 |
| *Fusarium equiseti* (Corda) Sacc. | 5 | 1 | 3 | - | 8 | 3 | 17 | 6 | 20 | 9 | 53 | 19 | 72 |
| *Fusarium oxysporum* Schl. | 13 | 4 | 14 | 4 | 8 | 2 | 18 | 7 | 24 | 9 | 77 | 26 | 103 |
| *Fusarium poae* (Peck) Woll. | 7 | 2 | 7 | 3 | - | - | 9 | 4 | 15 | 7 | 38 | 16 | 54 |
| *Fusarium sporotrichioides* Sherb. | 1 | - | 3 | - | 1 | 2 | 8 | 3 | 8 | 2 | 21 | 7 | 28 |
| *Mucor hiemalis* Wehmer | 5 | 2 | 2 | 2 | 3 | - | 1 | - | 6 | 1 | 17 | 5 | 22 |
| *Penicillium notatum* Westling | 7 | 3 | 7 | 2 | 8 | 3 | 2 | 2 | 5 | 2 | 29 | 12 | 41 |
| *Penicillium verrucosum* Dierckx var. *verrucosum* Samson, Stolk et Hadlok | 3 | - | 1 | - | - | - | 3 | - | 5 | 2 | 12 | 2 | 14 |
| *Rhizoctonia solani* Kühn | 5 | 1 | 7 | 2 | 4 | - | 11 | 5 | 15 | 8 | 42 | 16 | 58 |
| *Rhizopus nigricans* Ehrenberg | 4 | 1 | 1 | 2 | 1 | 1 | 5 | - | 6 | 3 | 17 | 7 | 24 |
| *Sclerotinia sclerotiorum* (Lib.) de Bary | 4 | 2 | 3 | - | 5 | - | 15 | 5 | 11 | 4 | 38 | 11 | 49 |
| **Total** | **85** | **22** | **74** | **21** | **58** | **20** | **126** | **40** | **160** | **64** | **503** | **167** | **670** |

***Table 5.*** *The number of bacteria and fungi in the rhizosphere of runner bean (mean from the years 2005-2006); *mean values in columns marked with the same letter do not differ significantly at $p \leq 0.05$.*

| Treatment | Total umber of bacteria (mln • g$^{-1}$ d. w. of soil) | Total number of Bacillus spp. (mln • g$^{-1}$ d. w. of soil) | Total number of Pseudomonas spp. (mln • g$^{-1}$ d. w. of soil) | Total number of fungi (thous. • g$^{-1}$ d. w. of soil) |
|---|---|---|---|---|
| Polyversum | 4.86$^{c}$ | 2.67$^{e}$ | 0.51$^{a}$ | 16.55$^{c}$ |
| Biochikol 020 PC | 4.51$^{c}$ | 1.53$^{c}$ | 1.57$^{c}$ | 15.28$^{b}$ |
| Biosept 33 SL | 5.74$^{d}$ | 1.92$^{d}$ | 1.56$^{c}$ | 11.29$^{a}$ |
| Zaprawa Oxafun T | 2.74$^{b}$ | 1.16$^{b}$ | 0.90$^{b}$ | 19.26$^{d}$ |
| Control | 1.98$^{a}$ | 0.59$^{a}$ | 0.46$^{a}$ | 26.25$^{e}$ |

Totally, 552 fungi isolates were obtained from the rhizosphere of runner bean growing in particular combinations (Table 6). The most frequently isolated fungi belonged to the genera of *Altenaria, Cladosporium, Fusarium, Penicillium, Rhizoctonia* and *Trichoderma. Genus Fusarium* was represented by *F. culmorum, F. oxysporum* and *F. solani,* while genus *Gliocladium* was represented by *G. catenulatum* and *G. roseum*. Such species as *T. harzianum, T. koningii* and *T. viride* occurred within *Trichoderma* spp. The proportion of species from genera *Gliocladium* and *Trichoderma* was higher in the rhizosphere of runner bean in the combinations with biopreparations as compared to the combination with fungicide or the control (Table 6). According to Transmo et al. [19], natural antagonists, including the fungi from genera *Gliocladium* and *Trichoderma,* show a high increase of biomass and production of chitinolitic enzymes in the environment containing chitosan. Therefore, the biopreparations introduced into the soil, including chitosan, could contribute to the increase of the populations of *Gliocladium* spp. and *Trichoderma* spp.

It was found out on the basis of laboratory studies that the biopreparations used in the experiment contributed to the increase of the number of antagonistis bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp. and *Trichoderma* spp.) in the rhizosphere of runner bean. The most antagonistic bacteria were obtained in the rhizosphere of runner bean after introducing Biosept 33 SL, and the most antagonistic fungi were obtained after using Biochikol 020 PC or Biosept 33 SL (Table 7).

***Table 7.*** *The number of antagonistic bacteria and fungi in the rhizosphere of runner bean (mean from the years 2005-2006).*

| Antagonistic bacteria and fungi | Treatment / Number of isolates | | | | |
|---|---|---|---|---|---|
| | Polyversum | Biochikol 020 PC | Biosept 33 SL | Zaprawa Oxafun T | Control |
| *Bacillus* spp. | 8 | 6 | 21 | 3 | 2 |
| *Pseudomonas* spp. | 40 | 14 | 28 | 9 | 4 |
| Mean | **48** | **20** | **49** | **12** | **6** |
| *Gliocladium* spp. | 11 | 15 | 12 | 4 | 2 |
| *Trichoderma* spp. | 19 | 29 | 31 | 6 | 6 |
| Mean | **30** | **44** | **43** | **10** | **8** |
| Total | **78** | **64** | **92** | **22** | **14** |

***Table 6.*** *Fungi frequently isolated from rhizosphere of runner bean (total from the years 2005-2006).*

| Fungus species | Treatment / Number of isolates | | | | | Total |
|---|---|---|---|---|---|---|
| | Polyversum | Biochikol 020 PC | Biosept 33 SL | Zaprawa Oxafun T | Control | |
| *Alternaria alternata* (Fr.) Keissler | 3 | 4 | 7 | 9 | 14 | 37 |
| *Aspergillus niger* van Tiegh | 5 | 4 | - | 6 | 10 | 25 |
| *Aureobasidium pullulans* (de Bary) Arnaud | 1 | - | 2 | 3 | 5 | 11 |
| *Botrytis cinerea* Pers. | 3 | 3 | - | 10 | 14 | 30 |
| *Cladosporium cladosporioides* Fres de Vries | 3 | 5 | 4 | 11 | 15 | 38 |
| *Epicoccum purpurascens* Her. ex. Schl. | - | - | - | 3 | 5 | 8 |
| *Fusarium culmorum* (W.G.Sm.) Sacc. | 6 | 4 | 3 | 7 | 8 | 28 |
| *Fusarium oxysporum* Schl. | 13 | 9 | 9 | 20 | 27 | 78 |
| *Fusarium solani* (Mart.) Sacc. | 3 | 2 | 3 | 9 | 15 | 32 |
| *Gliocladium catenulatum* Gilman Abbott | 8 | 10 | 8 | 2 | 1 | 29 |
| *Gliocladium roseum* Bainier | 3 | 5 | 4 | 2 | 1 | 15 |
| *Humicola grisea* Traaen | 5 | 1 | 2 | 4 | 5 | 17 |
| *Mucor globosus* Fischer | 1 | - | 2 | - | - | 3 |
| *Mucor mucedo* Fresenius | 3 | 2 | - | 4 | - | 9 |
| *Penicillium expansum* Link ex. S. F. Gray | - | - | 3 | - | - | 3 |
| *Penicillium chrysogenum* Thom | 4 | 3 | 1 | - | 2 | 10 |
| *Penicillium verrucosum* Dierckx var. *verrucosum* Samson, Stolk et Hadlok | 4 | 5 | 4 | 7 | 10 | 30 |
| *Rhizoctonia solani* Kűhn | 4 | 2 | - | 9 | 15 | 30 |
| *Rhizopus nigricans* Ehrenberg | 4 | 5 | 3 | 1 | - | 13 |
| *Talaromyces flavus* (Kőcker) Stolk et Samson | 1 | 3 | 2 | 6 | 3 | 15 |
| *Trichoderma harzianum* Rifai | 6 | - | 7 | 1 | 2 | 16 |
| *Trichoderma koningii* Oud. | 5 | 12 | 12 | 3 | 2 | 34 |
| *Trichoderma viride* Pers. ex. S.F. Gray | 8 | 17 | 12 | 2 | 2 | 41 |
| **Total** | **93** | **96** | **88** | **119** | **156** | **552** |

The effect of the biopreparations used in the experiment in limiting the development of fungi pathogenic towards runner bean and in stimulating the development of antagonistic fungi in the rhizosphere of this plant is first of all connected with the effect of biologically active substances. As reported by Pospieszny [16], chitosan found in Biochikol 020 PC not only induces the systemic resistance (SAR) acquired in plants but it also contributes to the increase of biomass and the production of chitinolitic enzymes in fungi from genus *Trichoderma*. The effect of Biosept 33 SL is connected with the presence of endogenous flavonoids of grapefruit and glycosides [3]. These compounds inhibit the germination of sporules, the growth of sprout hypha and vegetative hyphae through the damage of membrane systems and they also inhibit the activity of respiratory enzymes [2]. On the other hand, *Pythium oligandrum* contained in Polyversum can colonize the root system of plants and remain in the rhizsophere through the vegetation in this way hampering the colonization of this sphere by pathogens [4].

That is the reason why it can be supposed that abundant occurrence of antagonists in the rhizsophere of the studied plant after introducing biopreparations could limit the development of plant pathogens and effectively protect the plants of runner bean from infection by soil-borne fungi. This fact is confirmed for example by the studies conducted by Orlikowski [8], Orlikowski and Jaworska-Marosz [9], Orlikowski and Skrzypczak [11] and Patkowska et al. [14].

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