

SURVIVAL RATE, INSECTICIDAL AND FUNGISTATIC ACTIVITY OF ANTAGONISTIC ACTINOMYCETE *STREPTOMYCES GRISEOVIRIDIS* AND ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* IN SEPARATE AND COMBINED INTRODUCTIONS TO THE SOIL

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Abstract: Highly active antagonistic actinomycete *Streptomyces griseoviridis* and entomopathogenic fungus *Beauveria bassiana* were applied to the soil separately and together (in association) in the laboratory experiments. We assessed survival rate, insecticidal and fungistatic activity of these strains. We also tested the influence of synthetic insecticide Regent 25[®] (fipronil 25g/l) on investigated parameters. Additionally, insecticidal activity of both strains was compared with insecticidal activity of Regent. It was shown that both strains, especially *S. griseoviridis*, good survived in soil. Population density of *S. griseoviridis* in the association with *B. bassiana* increased 2–3 times compared to initial density. Regent considerably reduced population density of *S. griseoviridis* and *B. bassiana*. Insecticidal efficiency of *S. griseoviridis* was comparable with the effect of synthetic insecticide Regent and reached 89.2% and 86.8% respectively. Fungistatic activity towards *Fusarium oxysporum* showed only *S. griseoviridis* and it was observed that this activity decreased in time course.

Key words: *Streptomyces griseoviridis*, *Beauveria bassiana*, *Fusarium oxysporum*, fungistatic effect, insecticidal activity, bioinsecticides

INTRODUCTION

The rhizosphere of many species of plant is the main source of actinomycetes, especially *Streptomyces* (Williams et al. 1989; Cao et al. 2005). *Streptomyces* are also

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the natural source of many active substances with a broad range of action, including these with fungistatic properties (Lockwood 1959; Crawford et al. 1993; Samac et al. 2003). Thanks to that, similarly as entomopathogenic fungi used for pests control, antagonistic microorganisms are used as biopesticides in biological methods of protecting plants against diseases (Bochow 1989; Lahdenpera et al. 1991; Kandybin and Smirnov 1996; Shoda 2000; Minuto et al. 2006). Lately, there is more and more interest in complex biopesticides consist of different biological control agents which result in higher effectiveness or both fungistatic and insecticidal effect (Lobanok and Kolomiets 2000; Jung 2005; Yi and Ehlers 2005).

Concerning this, the aim of presented studies was the attempt to using highly active strain of antagonistic bacteria *Streptomyces griseoviridis* (Anderson et al. 1956) together with the strain of entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill for control of pests and plant pathogens. Survival rate and the fungistatic and insecticidal activity were checked for both strains, after the combined introduction to the soil.

MATERIALS AND METHODS

The strain *S. griseoviridis* (BIM B 264 Str+R) come from the collection of the Institute of Microbiology, BNAS in Minsk. It was raised to the experimental researches in a incubator in 28°C on the medium of the following composition (g/l): oat flour – 20, NaCl – 3, CaCO₃ – 3, peptone – 5, corn extract – 1, agar – 20, pH 7.0–7.2.

The strain *B. bassiana* (23) was from the collection of the Institute of Ecology PAS (currently CER PAS) at Dziekanów Leśny. To the experimental studies the strain *B. bassiana* was raised in a incubator at 24°C on the Sabouraud's medium (g/l) consisted of: glucose – 40, peptone – 10, agar – 20.

Survival rate and insecticidal activity of *S. griseoviridis* and *B. bassiana* strains were tested in the soil by microcosmic experiments in the laboratory conditions. The soil in Petri dishes (30 g fresh soil) was sterilized three times with the 24 h breaks. After the sterilization the soil in the dishes was moistured to 30% RH by adding distilled water and suspension of spores of *B. bassiana* and *S. griseoviridis*. The received concentration of spores was equal to 2.3×10^7 /g of the soil.

Monitoring of the survival rate of examined strains in soil was carried out using antibiotic-resistant mutants. For *S. griseoviridis* antibiotic resistance was received on streptomycin in concentration of 500 µg/ml and for *B. bassiana* on nystatin in concentration of 500 µg/ml (Egorov 1986). Survival rate of tested strains after combined introduction to the soil (in so called association) was checked every 2–3 days over 3 weeks by plating *S. griseoviridis* on the medium with oat flour, whereas *B. bassiana* on Sabouraud's medium (the selective factor was the antibiotics in concentrations against which the examined strains gained antibiotic resistance). Population density of *S. griseoviridis* and *B. bassiana* after separate introduction to the soil (without association) was served as the control. There were 6 replications on each treatment of the experiment. The results are presented as mean of Colony Forming Units (CFU) per gram of dry soil (Pepper et al. 1995).

Insecticidal activity of the strains *B. bassiana* and *S. griseoviridis* in the association and after separate introduction to the soil was tested on a laboratory insect – *Galleria mellonella* L. (*Lepidoptera*) larvae. Ten larvae were put (in 6 replications) into a Petri dish containing the soil with introduced strains of the examined microorganisms.

Mortality rate of the insects was checked every 1–2 days for two weeks. Mortality of *G. mellonella* larvae in the soil without the introduced strains was served as the control. Level of insect's mortality in each treatment of the experiment and in the control is expressed as percentage value and was calculated by using Abbot's formula which incorporated natural control mortality.

Fungistatic activity of the examined strains *B. bassiana* and *S. griseoviridis* in the association and after separate introduction to the soil was checked in relation with phytopathogenic fungus *Fusarium oxysporum* (Schltdl.) by diffusion in agar method (wells method) (Szegi 1983). Four-days old liquid culture of *F. oxysporum* (1 ml) was added to 4 ml of potato dextrose medium (PDA) (1.2% w/v agar) and poured on a Petri dish containing previously prepared PDA medium (2% w/v agar). In such prepared medium, holes were made and about 300 µg of sterilized soil (control) or the soil with the introduced strains of *B. bassiana* and *S. griseoviridis* (Fig. 1) was inserted.

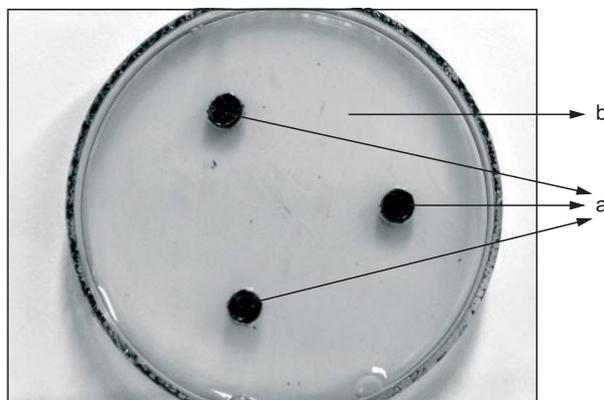


Fig 1. Estimation of fungistatic activity of the examined strains – the wells method
 a – soil (in holes in the medium) with the introduced strains or sterilized soil (control)
 b – medium with suspension of test-fungus *F. oxysporum*

Antagonism of the examined strains was evaluated on the basis of inhibition zones (in mm) of the test-fungus *F. oxysporum*, 5, 7 and 10 days after introduction to the soil. The results are presented as an average of the three replications \pm SE.

Insecticidal activity of *B. bassiana* and *S. griseoviridis* was compared with synthetic insecticide Regent 25[®] (fipronil 25g/l) (Aventis, Germany) which was used in field dose (10l/100m²). The influence of this insecticide on survival rate and fungistatic activity of the examined strains in the soil were also tested.

RESULTS

Population density of the strain *S. griseoviridis* in the association with *B. bassiana* and after separate introduction (control) was at the same level between the 3th and the 5th day as it was introduced to the soil (i.e. on the level 10⁷ CFU/g of the soil). Between the 7th and the 14th day after introduction to the soil the population of

S. griseoviridis in the association with *B. bassiana* increased 2–3 times compared to initial density (Table 1). CFU-s of *B. bassiana* in the association with *S. griseoviridis* after 7 days after introduction to the soil were 3–17 times lower in comparison with the control (Table 1).

Table 1. Dynamics of population density of *B. bassiana* and *S. griseoviridis* after separate and combined introduction to the soil and effect of Regent insecticide (initial density of *B. bassiana* and *S. griseoviridis* spores in each treatments was equal to 2.3×10^7 /g soil)

Treatments	Population density [CFU x 10 ⁷ /g soil/day]								
	3	5	7	10	12	14	17	19	21
<i>B.b.</i> (control)	0.36	0.52	0.78	0.93	1.0	0.45	0.38	0.14	0.14
<i>S.g.</i> (control)	0.22	7.6	15.0	18.0	10.0	8.3	7.7	5.5	3.3
<i>B.b.</i> + R	0.012	0.006	0.006	0.005	0.011	0.008	0.007	0.008	0.005
<i>S.g.</i> + R	0.85	1.4	1.5	1.3	0.91	0.78	0.78	0.78	0.74
<i>B.b.</i> in association with <i>S.g.</i>	0.11	0.44	0.046	0.056	0.068	0.057	0.057	0.046	0.046
<i>S.g.</i> in association with <i>B.b.</i>	2.1	2.7	6.0	5.3	7.1	7.9	3.9	2.1	1.2
<i>B.b.</i> in association with <i>S.g.</i> + R	0.009	0.011	0.008	0.008	0.011	0.011	0.009	0.009	0.008
<i>S.g.</i> in associations with <i>B.b.</i> + R	0.7	0.8	0.7	0.46	0.6	0.41	0.49	0.48	0.87

B.b. – *Beauveria bassiana*

S.g. – *Streptomyces griseoviridis*

R – Regent insecticide

The population dynamics of the examined strains *B. bassiana* and *S. griseoviridis* decreased 10–200 times after introducing Regent insecticide to the soil; however, *B. bassiana* strain was more sensitive than *S. griseoviridis*. It occurred also in the association of both microorganisms.

Studies of the mortality of test-insects *G. mellonella* showed that the strain *S. griseoviridis* had higher insecticidal activity by comparison to *B. bassiana*. However, the greatest mortality of insects was noticed in the association of the examined fungus and actinomycete, which indicated synergistic action of both strains (Fig. 2).

Mortality of *G. mellonella* caused by Regent insecticide was similar to mortality caused by *S. griseoviridis*. However, in the first two days of the experiment Regent insecticide killed larvae *G. mellonella* considerably faster than the examined strains. Regent insecticide was more effective than entomopathogenic fungus *B. bassiana*, whereas in comparison with the association of *B. bassiana* and *S. griseoviridis* it caused lower mortality of test insects by almost 10% (Fig. 2).

The strain *B. bassiana* did not show any fungistatic activity. The test-fungus *F. oxysporum* covered all the surface of a dish, not omitting zones around the soil with introduced strain *B. bassiana* (Fig. 3a).

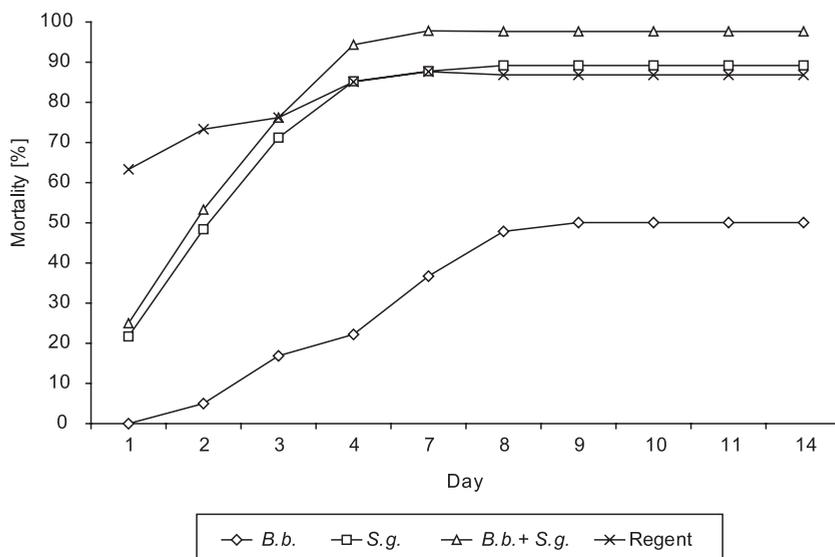


Fig. 2. Mortality of test-insect *G. mellonella* in the soil with the separate and combined introduced strains of *B. bassiana* (*B.b.*) and *S. griseoviridis* (*S.g.*) to the soil in the comparison with Regent insecticide

Until 7th day the strain *S. griseoviridis* after separate introduction to the soil characterized by high fungistatic activity (Fig. 3b and Fig. 4) against *F. oxysporum*. Fungistatic activity of *S. griseoviridis* in the association with *B. bassiana* was high until 5th day after introduction to the soil. However, this activity decreased in time (Fig. 4). After 10 days the strain *S. griseoviridis* showed similar fungistatic effect both: separately and in the association with *B. bassiana* (Fig. 4).

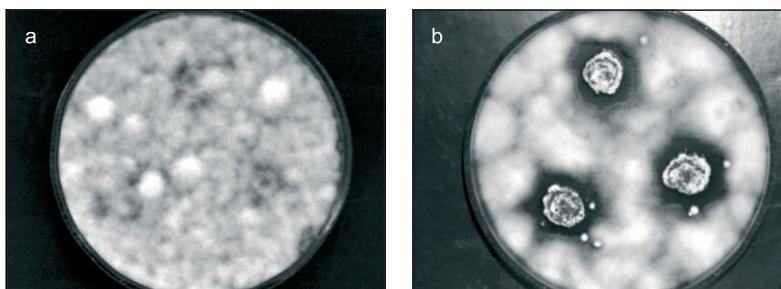


Fig. 3. Fungistatic activity of *B. bassiana* (a) and *S. griseoviridis* (b) strains against test-fungus *F. oxysporum*

Fungistatic activity of strain *S. griseoviridis* and its association with *B. bassiana* in presence of synthetic Regent insecticide 5 days after the introduction was lower in comparison to the experiment without Regent and after 7 and 10 days remained at the same level. Because of fungistatic activity of *S. griseoviridis* decreased in course of time, 10 days after introduction to the soil its fungistatic activity was at the same level as in treatments with Regent (Fig. 4).

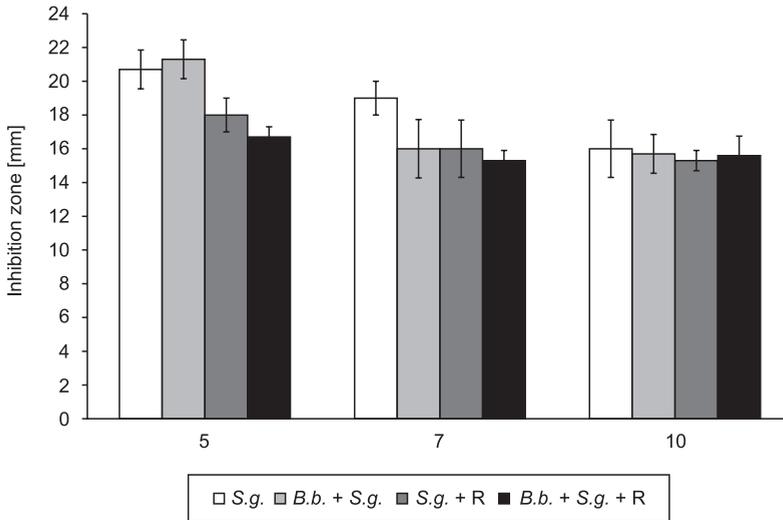


Fig. 4. Effect of Regent insecticide (R) on fungistatic activity of *S. griseoviridis* (*S.g.*) and its association with *B. bassiana* (*B.b.*) against test-fungus *F. oxysporum*

DISCUSSION

Current studies on interactions between *B. bassiana* and *S. griseoviridis* in the soil confirmed results of our previous experiments carried out on the synthetic media in which we tested the influence of antagonistic microorganisms on the growth of entomopathogenic fungi (Popowska-Nowak et al. 2003). In these experiments it was found that the genus *Streptomyces* did not show any strong antagonism towards entomopathogenic fungi.

The results of current experiments also showed that the tested strains *B. bassiana* and *S. griseoviridis*, introduced together to the soil, survived and remained at the quite high level in 21 days. As Kolomiets et al. (1999) proved that actinomycete of the genus *Streptomyces* took advantage of different kinds of energy sources, both mineral and organic, therefore their survival rate should be high in different types of soil. In the mentioned article authors stated that the strain 6GL *S. griseoviridis* remained on the high level in the soil for 4 months and kept its fungistatic high activity against *F. oxysporum* (Kolomiets et al. 1999).

Commonly used pesticides have an influence on the growth and activity of all kinds of microorganisms in the soil, as also showed the presented results. Regent insecticide decreased population density of the examined strains and *B. bassiana* was more susceptible than *S. griseoviridis*. A lot of researches concerning the influence of insecticides also indicate that susceptibility of both fungi, including entomopathogenic ones, and bacterium on these agents may differ not only among different species, but also among different strains (Martinez-Toledo et al. 1922; Das and Mukherjee 2000). Pędziwilk (1995) found that pesticides not only reduced population density of actinomycete, but also their fungistatic activity. Similarly in our researches, in the presence of Regent insecticide fungistatic activity of *S. griseoviridis* was lower than in variants without Regent (until the 7th day).

Unexpectedly, in the presented studies the strain *B. bassiana* had lower insecticidal

effectiveness after introducing to the soil in comparison to *S. griseoviridis* and synthetic Regent insecticide. However, the association of both strains resulted in highest mortality of test insects. Similar effect obtained Wraight and Ramos (2005) using commercial biopesticide based on the fungal pathogen *B. bassiana* and the bacterial pathogen *Bacillus thuringiensis* subsp. *tenebrionis*. *B. bassiana* and *B. thuringiensis* were applied alone and in combination against larval populations of the Colorado potato beetle, *Leptinotarsa decemlineata*. Both pathogens applied together gave greater reduction in larval population than the two biopesticides acted independently.

In accordance with obtained results the strain *S. griseoviridis* displays much higher antagonistic effect towards plant pests and fungal pathogens compared to *B. bassiana*. Taking into account this fact and economical aspect *S. griseoviridis* can be recommended to be used as mono component biopreparations. In conclusion, it seems that biological control using antagonistic microorganisms, such as *Streptomyces*, to suppress plant diseases and pests offers a powerful alternative to use of synthetic chemicals.

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POLISH SUMMARY

OCENA PRZEŻYWALNOŚCI, AKTYWNOŚCI OWADOBÓJCZEJ I FUNGI-STATYCZNEJ ANTAGONISTYCZNEGO PROMIENIOWCA *STREPTOMYCES GRISEOVIRIDIS* I ENTOMOPATOGENICZNEGO GRZYBA *BEAUVERIA BASSIANA* PRZY ODDZIELNEJ I ŁĄCZNEJ INTRODUKCJI DO GLEBY

W eksperymentach laboratoryjnych oceniano przeżywalność, owadobójczą i fungistatyczną aktywność szczepu antagonistycznego promieniowca *Streptomyces griseoviridis* i grzyba owadobójczego *Beauveria bassiana* wprowadzonych do gleby oddzielnie i łącznie w tzw. asocjacji. Oceniano również wpływ syntetycznego insektycydu Regent wprowadzonego do gleby jednocześnie z badanymi szczepami na testowane parametry. Dodatkowo porównano owadobójczą aktywność obu szczepów z owadobójczą aktywnością preparatu Regent 25[®]. Stwierdzono, że oba szczepy, a w szczególności *S. griseoviridis* dobrze przeżywały po wprowadzeniu do gleby. Liczebność *S. griseoviridis* w asocjacji z *B. bassiana* wzrastała w czasie trwania eksperymentu 2–3-krotnie w porównaniu do początkowej liczebności. Natomiast Regent znacząco obniżał liczebność badanych szczepów w glebie. Owadobójcza aktywność *S. griseoviridis* była porównywalna z aktywnością owadobójczą preparatu Regent. Śmiertelność owadów testowych wyniosła odpowiednio 89,2% i 86,8%. Fungistatyczną aktywność w stosunku do *F. oxysporum* wykazywał tylko *S. griseoviridis*. Obserwowano spadek tej aktywności w trakcie trwania eksperymentu.