

THE ANTAGONISTIC ACTIVITY OF ACTINOMYCETES OF *STREPTOMYCES* GENUS IN RELATION TO *TRICHODERMA KONINGII*

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Received: 2015.11.08

Accepted: 2015.12.09

Published: 2016.01.06

ABSTRACT

The aim of the study was to estimate the effect of actinomycetes of genus *Streptomyces* on the growth of the antagonistic fungus *Trichoderma koningii*. 150 strains of *Streptomyces* spp. isolated from two potato cropping systems were used to the tests. Analyses were conducted experimentally *in vitro* on PDA medium with pH 6 and 7. The results obtained clearly indicate the inhibitory effect of actinomycetes on the fungus *T. koningii*. Of the tested population of actinomycetes only two strains did not inhibit the growth of the tested fungus. The strains from the rhizosphere of potato cultivated under the ecological system were predominant in the group of actinomycetes with a very strong effect. pH value appeared to be a crucial factor influencing the inhibition zone. High antagonistic activity in relation to the useful fungus *T. koningii* noted with numerous strains of *Streptomyces* spp. is unfavorable in respect of biological control.

Keywords: antagonism, *Streptomyces*, *Trichoderma koningii*, soil, antifungal activity.

INTRODUCTION

The main problem connected with using microorganisms in biological control is their ability to survive in soil after introduction, and their effective action. There are many biological and physicochemical factors determining the survival of microorganisms introducing into soil. Of the biological factors, native bacteria, fungi, actinomycetes and their metabolites (antibiotics, mycotoxines, enzymes) are of great importance [Oskay 2009, Sharma et al. 2014].

Actinomycetes are the most known microorganisms with antagonistic properties in consideration of the synthesis of antibiotics. This most numerous group of antibiotic producers can protect plants against phytopathogens, which was noted in many field and laboratory experiments [Kumar et al. 2010, Evangelista-Martínez 2014, Kamal and Sharma 2014]. Antagonistic abilities of *Streptomyces* result also from producing extracellular enzymes causing the lysis of other microorganisms. They may parasitize on the hyphae of fungi, their oospores, or sclerotia [Prapagdee et

al. 2008]. Mycoantagonistic properties of strains *Streptomyces* spp. were generally studied in relation to phytopathogenic fungi, e.g. *Rhizoctonia solani*, *Fusarium solani*, *Phytophthora* sp. [Breza-Boruta et al. 2004, Bressan and Figueiredo 2008, Al-Askar et al. 2011], while little is known about their effect on potentially antagonistic fungi.

The best known antagonists of microorganisms causing plant diseases among soil fungi are *Trichoderma* genus, and in particular, the species: *T. viride*, *T. koningii*, *T. harzianum*. The mechanism of their action consists in the ability to synthesize antibiotics and possibility of specific parasitizing on the fungal spawn of phytopathogens by excreting an active complex of enzymes [Howell 2003, Gajera et al. 2012, Reddy et al. 2014]. Several preparations were developed based on these fungi. However, it appears that their introduction into soil not always results in the effective reduction in pathogens [Świerczyńska et al. 2011]. The reason for this may be the action of other microorganisms with stronger antagonistic properties [Compant et al. 2005, Felice et al. 2014].

Therefore, the aim of the study was to find out the inhibition rate of actinomycetes of *Streptomyces* genus on the growth of *Trichoderma koningii* mycelium. In this study we compared the antagonistic activity of the strains of *Streptomyces* spp. isolated from two potato plantations with different farming systems, ecological and conventional. The effect of the medium pH on the growth of the fungal mycelium has also been tested.

MATERIAL AND METHODS

150 strains of actinomycetes of the genus *Streptomyces* isolated from potato crop were used for the study. "Aster" potato was cultivated in two farming systems, ecological and conventional, on lessive soils. Detailed characterization of the specifics concerning cultivation technologies applied in the compared farming systems was presented in the study by Breza-Boruta [2013]. 75 strains were isolated during growth periods from each farming system: 30 strains from soil beyond roots (ZEG – ecological farming and ZTG – conventional farming), 30 from rhizosphere (ZER – ecological farming and ZTR - conventional farming) and 15 from potato rhizoplane (ZERp – ecological farming and ZTRp – conventional farming). Actinomycetes were isolated on the Williams and Davies selective medium [1965]. Diagnostic tests of actinomycetes strains were performed based on macro and microscopic features according to Bergey's Manual of Systematic Bacteriology [Goodfellow et al. 2012] and Crawford et al. [1993].

The tested fungus was the saprophytic species *Trichoderma koningii*, which had previously been examined for mycoparasitism abilities in relation to phytopathogenic fungi. *T. koningii* was isolated from the rhizosphere of potato cultivated on the ecological farm. Taxonomic determination of the fungus was made based on mycological keys, according to Domsch et al. [1980].

The method of two simultaneous cultures was used to estimate antagonistic features of investigated actinomycetes in relation to the tested fungus. Five-day-old cultures of *Streptomyces* spp. were used in the study. The cultures were inoculated with a line of 40 mm in length on potato dextrose agar (PDA, Merck). After 7 days' incubation, a 72-hour-old culture of *T. koningii* was added to each actinomycetes. The mycelium was placed in a form of circles of 8 mm in diameter. Control plates without actinomycetes were inocu-

lated in the same way. The experiment was conducted with three replications on two media with pH values 6 and 7.

The growth inhibition rate of *T. koningii* was estimated after 72 and 120 hours of incubation at 19–20 °C. The measure of inhibition rate was the difference between the radius of the fungal mycelium on the control plates with a given pH value and the radius of the fungal mycelium grown in the presence of actinomycetes, expressed in mm.

The evaluation of antagonistic properties of tested population of *Streptomyces* spp. in relation to *T. koningii* was performed using the modified method of Lee and Hwang [2002]. The effect of strains was characterized on a scale of five degrees, where the length of inhibition zone of the fungal mycelium was estimated proportionally, in relation to the control:

- 0% – lack of effect,
- 1 – 25% – weak effect
- 26 – 50% – moderate effect
- 51 – 75% – strong effect
- 76 – 100% – very strong effect.

The results were calculated statistically with the use of variance analysis. The differences of mean inhibition zones were evaluated based on Tukey's test ($p=0.05$) using Statistica 10.0 (StatSoft, Poland). The measurement of the fungal mycelium radius length after 5 days of cultivation was used for calculations.

RESULTS

The analyses showed strong antagonistic properties of the investigated population of actinomycetes in relation to the tested fungus. Only two strains of *Streptomyces* spp. (isolated from soil) did not inhibit the growth of *T. koningii*. The analysis with Student's t-test has shown a significant impact of pH value on the size of inhibition zones (Figures 1–3). In most cases, significantly larger zones were recorded on the medium with pH 6 than on that with pH 7.

Of actinomycetes isolated from soil, the strains derived from the ecological farming system proved to be slightly more active than those from the conventional farming (Figure 1). 27% of strains from the ecological farming and 13% from the conventional system were in the group of *T. koningii* very strongly inhibiting the fungal growth in the tests on the medium with pH 6. In the tests conducted on the medium with pH 7,

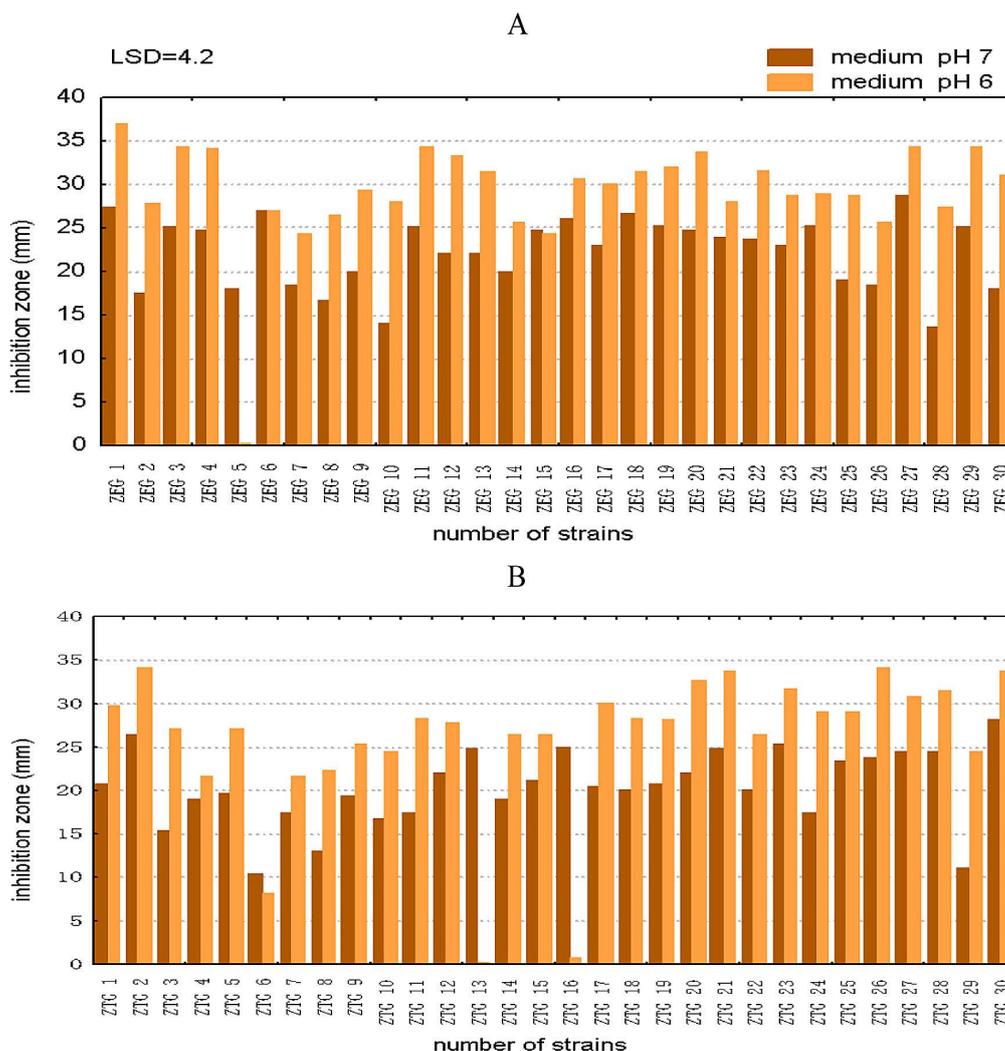


Figure 1. Influence of *Streptomyces* spp. strains isolated from non-rhizosphere soil of potato cultivated in ecological (A) and conventional (B) farming, on growth inhibition of *T. koningii*

13% and 10% of isolates, respectively, showed very strong inhibitory effect. Most isolates from soil (77%) strongly affected the growth of *T. koningii*. Only actinomycetes derived from the conventional farming system had a weak effect – 7% of strains tested on the medium with the lower pH value. Only 3% of strains, both from the conventional and ecological systems (on the medium with pH 6) showed the lack of inhibitory effect on the growth of *T. koningii*.

All the tested population of actinomycetes isolated from the rizosphere of potato cultivated under the ecological farming inhibited the growth of *T. koningii* (Figure 2). However, in the collection of *Streptomyces* spp. from the conventional system 10% of strains weakly inhibited the growth of the fungal mycelium were detected, but only on the medium with pH 6.7% of strains from both farming systems showed the moderate inhibitory effect. Very strong antagonism was detected in

33% of tested strains tested on the medium with the lower pH value and in 53% of strains growing on the medium with the higher pH (Figure 4).

Isolates derived from the rhizoplane of potato were also characterized by strong mycoantagonistic properties (Figure 3).

Totally 73% of strains from the ecological farming and 87% of strains coming from the conventional farming grown on the medium with pH 6, and 73% and 60%, respectively, grown on the medium with pH 7, which belong to the group strongly and very strongly inhibiting the growth of *T. koningii*. The other cultures of actinomycetes isolated from the surface of potato roots were marked by the moderate inhibitory activity (Figure 4).

Of the tested *Streptomyces* spp., the following strains showed the highest antagonistic activity against *Trichoderma koningii*: ZER-9 and ZER-29 – inhibiting the growth of the myce-

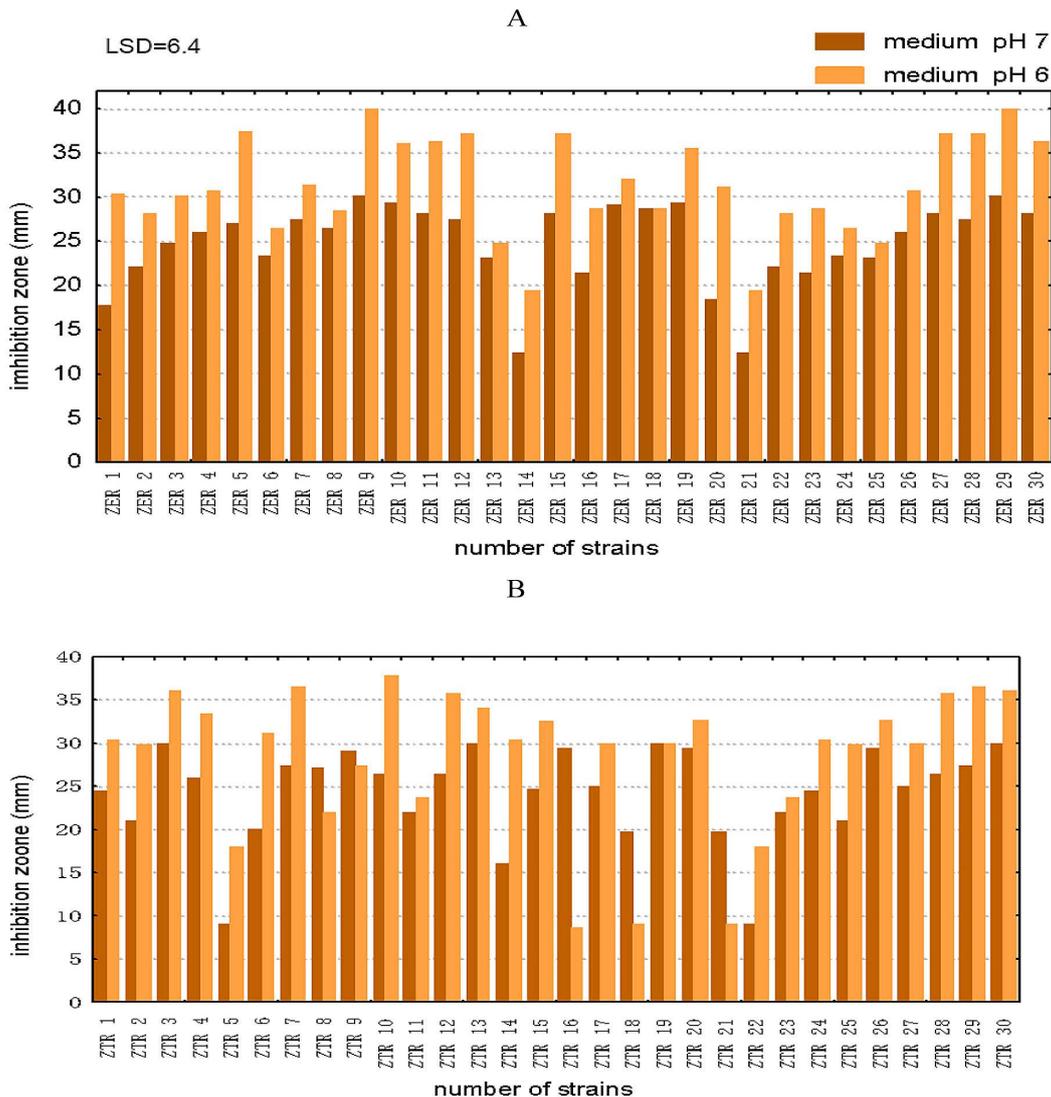


Figure 2. Influence of *Streptomyces* spp. strains isolated from rhizosphere of potato cultivated in ecological (A) and conventional (B) farming, on growth inhibition of *T. koningii*

lium to 40 mm; ZEG-1, ZER-5, ZER-12, ZER-15, ZER-27, ZER-28, ZTR-10 – inhibiting the growth to 37mm.

DISCUSSION

The present investigations indicate that actinomycetes can very actively inhibit the growth of useful fungi of the genus *Trichoderma*. The group of the most active actinomycetes very strongly inhibiting the growth of *Trichoderma koningii* included 50 of 150 strains tested. Oskay [2009] reports that fungi show much more resistance to antibiotics excreted by actinomycetes than bacteria. The presented results concerning the resistance of *T. koningii* do not confirm this. Their strong inhibitory properties could be observed also in other investigations on the antagonistic activity of

actinomycetes in relation to the fungi *Rhizoctonia solani* [Breza-Boruta 2004].

The phenomena of antagonism, irrespective of the nature of their mechanism, regulate the co-existence of various species of microorganisms, both pathogens and saprophytes. Both the fungi of the genus *Trichoderma* and actinomycetes with the antagonistic effect are good competitors and producers of antibiotics and enzymes [Solecka et al. 2013, Reddy et al. 2014].

The results obtained in our studies indicated that actinomycetes of the genus *Streptomyces* showed stronger antagonistic properties *in vitro* in comparison with *T. koningii*. Only two tested isolates, one from the ecological system (the strain ZEG-5) and one from the conventional system (ZTG-13), did not show antagonistic properties. The visible growth of the fungal mycelium on the culture of actinomycetes took place in these two

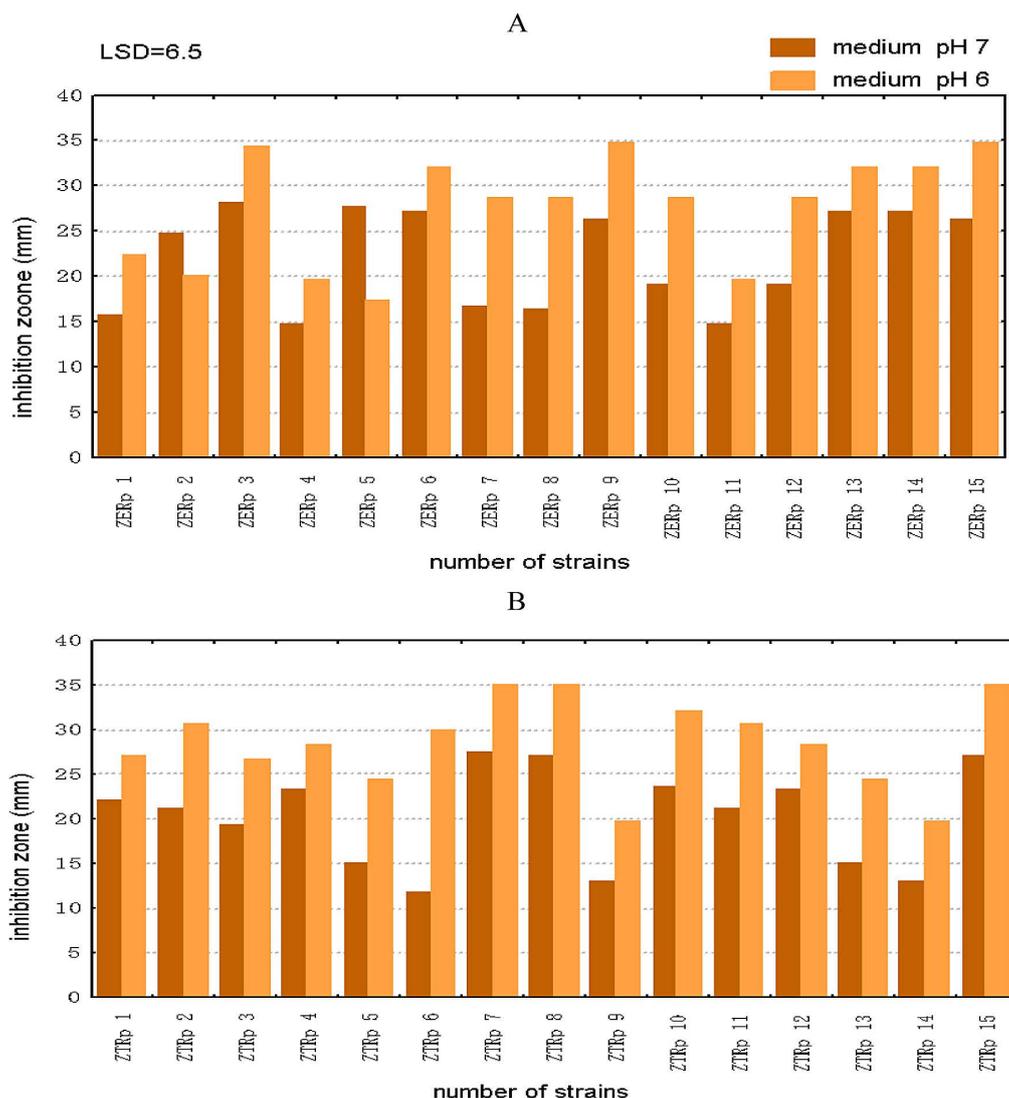


Figure 3. Influence of *Streptomyces* spp. strains isolated from rhizoplane of potato cultivated in ecological (A) and conventional (B) farming, on growth inhibition of *T. koningii*

cases. In opposition to mycoparasitizing, antibiosis is more effective in relation to metabolically active structures. In general, it does not require direct contact with a sensitive fungus and does not lead to destruction of its cells [Prapagdee et al. 2008, Sharma et al. 2014].

Differentiated effect of antagonistic bacteria on antagonistic fungi was observed by Pięta and Patkowska [2003]. The results of their studies indicated the low sensitivity of *Trichoderma koningii* and *Trichoderma viride* to the presence of the tested bacteria. Inhibitory effect occurred in the case of common growth of fungi with *Bacillus* sp., while the bacteria *Erwinia* sp., *Pseudomonas* sp. and *Bacillus mycoides* did not show any influence.

The strains of *Streptomyces* spp. were marked by intensive production of metabolites and their diffusion into the medium, which caused a con-

siderable inhibition of the mycelium growth. The genus *Streptomyces* is known for production of many antibiotics, including fungicides. They synthesize more than 2000 antibiotics, which makes 82.5% of their production by actinomycetes [Dharmaraj 2010, Solecka 2013]. The ability to biosynthesize antibiotics is limited to very specific ecological niches. Micro-amounts of the antibiotics produced by actinomycetes in these habitats are big enough for them to act effectively to other microorganisms [Schrey and Tarkka 2008, Felice et al. 2014].

Marcinowska and Bis [1997] report that such cultivated plants as potato and flax affect negatively the growth of antagonistic actinomycetes. The results of the present studies do not confirm such a negative influence in case of potato. The study proved a high activity of actinomycetes derived from potato cultivation

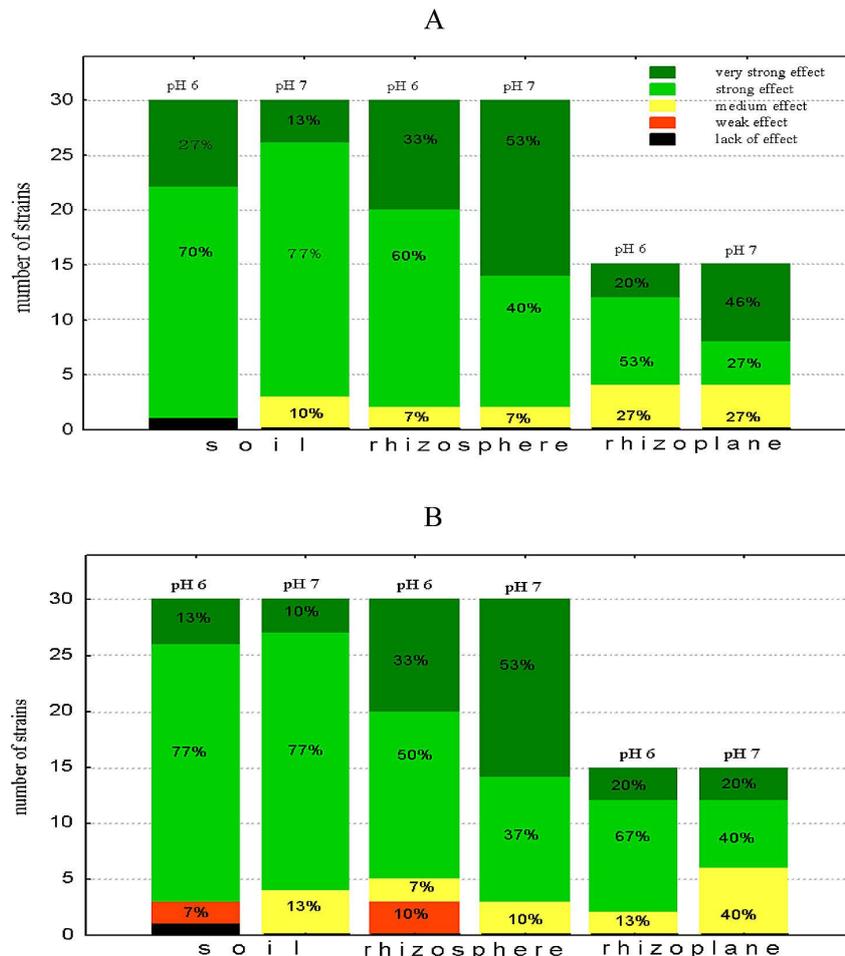


Figure 4. The number of *Streptomyces* spp. strains isolated from the soil, rhizosphere and rhizoplane of potato cultivated in ecological (A) and conventional (B) farming, and strength of their influence on the growth of *Trichoderma koningii*

in relation to *T. koningii*. Also in other studies a collection of actinomycetes showed antagonistic properties in relation to pathogenic fungi [Brezza-Boruta et al. 2004].

Differentiation in the antagonistic activity of actinomycetes, according to the place of isolation, was recorded (soil beyond roots, rhizosphere and rhizoplane). The most active were the strains coming from potato rhizosphere. This can prove the favourable influence of potato roots excretions on the antibiotic activity of *Streptomyces* spp. [Pięta and Patkowska 2003, Mandić et al. 2011].

Of the tested population of *Streptomyces* spp., the strains isolated from the ecological farm showed a higher inhibitory strength in relation to *T. koningii*. Based on the obtained results, it may be suggested that more favourable conditions for the development of antagonistic actinomycetes can be found in the ecological farming than in a conventional system. There are not any comparisons concerning the activity of actinomycetes

coming from different farming systems in the available bibliography.

Both the tested strains of *Streptomyces* spp. and the investigated fungus come from the same environment. The strong antagonism of the strains *Streptomyces in vitro* is very likely to have an inhibitory effect on *T. koningii* in soil. We should regard physicochemical soil properties influencing the survival rate of potential antagonists. The most important of them are the structure of soil, temperature, pH value, the content of nutrients and the presence of toxic chemical compounds [Mandić et al. 2011, Felice et al. 2014]. According to Singh et al. [2014], species of the genus *Trichoderma* are not very sensitive to the effect of different environmental factors and develop in the wide range of temperatures and the medium pH values. From the present study it appears that *T. koningii* is sensitive to the change in pH value. Considerable differences in the fungal mycelium radius length were obtained both on the control plates and in

a culture with actinomycetes. Significantly larger inhibition zones were indicated on the medium with pH 6 than on that with pH 7, which has been confirmed by statistic calculations.

It is worth of notice, which was also observed by many investigators [Compant et al. 2005, Evangelista-Martínez 2014, Kamal and Sharma 2014], that it is very difficult to select different microorganisms for biological control. The most essential is that potential antagonists may be ecologically competitive in respect of the interaction between plants and pathogens both in soil and in phyllosphere.

CONCLUSIONS

1. The actinomycetes of genus *Streptomyces* proved to be strong antagonists in relation to the fungus *Trichoderma koningii*. Of the strains limiting the growth of the fungal mycelium, the following strains isolated from potato rhizosphere were predominant: ZER-9, ZER-29, ZER-5, ZER-12, ZER-27, ZER-28, ZTR-10).
2. Significant influence of the medium pH on the growth of the mycelium of *T. koningii* was recorded both on the control plates and in the culture with actinomycetes. Statistically significantly larger inhibition zones were obtained on the medium with pH 6 than on the medium with pH 7.
3. Of the tested strains, the actinomycetes isolated from the ecological potato farming system showed stronger antagonistic properties, in comparison with the isolates derived from the conventional farming system.
4. From the point of view of biological control, strong inhibiting of growth of the fungus *Trichoderma koningii* (a potential mycoparasite of numerous plant pathogens) by so many strains of *Streptomyces* spp. is an undesirable phenomenon.

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