

THE USE OF *BACILLUS SUBTILIS* FOR THE PROTECTION OF POTATO AGAINST *PHYTOPHTHORA INFESTANS* IN ORGANIC FARMING

Summary

This study includes 1) greenhouse tests to evaluate *B. subtilis* used as preventative and intervention treatments against *P. infestans* on potato plants and 2) field studies. Lord and Denar cultivars were used. As prevention treatments, the Serenade ASO (contains *Bacillus subtilis*) was applied at a dose of 8 l / ha as 3 and /or 6 treatments at intervals of 7 days. The first treatment was performed at BBCH 11, symptoms of *P. infestans*, *A. solani*, *A. alternata* were not observed on the plants. As a reference, copper-based fungicide (Nordox 75 WG) treatments were used at a total dose of 3 kg Cu/ha / season. Over the season, observations were made concerning the occurrence of disease symptoms (potato blight, alternaria) that caused plants' drying up. Effectiveness of the treatments was evaluated by assessing the percentage of infestation of potato plants, in relation to the combination of experiments. It has been found that *B. subtilis* has the potential to protect against *P. infestans* used as preventive application, while in field conditions the protective effect occurs only after 6 treatments and is comparable to that of copper.

Key words: beneficial bacteria, potato cultivars, preventive treatments, late potato blight

WYKORZYSTANIE *BACILLUS SUBTILIS* DO OCHRONY PRZED *PHYTOPHTHORA INFESTANS* W UPRAWACH EKOLOGICZNYCH

Streszczenie

W ramach badań wykonano 1) badania szklarniowe w celu oceny zastosowania prewencyjnego i interwencyjnego *B. subtilis* w stosunku do *P. infestans* na roślinach ziemniaka oraz 2) badania polowe. Wykorzystano odmiany Lord i Denar, odpowiednio. Jako prewencję zastosowano środek Serenade ASO w postaci drobnokropelkowego opryskiwania nalistnego w dawkach 8l/ha. Wykonano 3 i/lub 6 zabiegów w odstępie 7 dni. Pierwszy zabieg wykonano w fazie BBCH 11. W momencie rozpoczęcia zabiegów nie zanotowano obecności *P. infestans* i *A. solani*, *A. alternata* na roślinach. Jako referencję zastosowano zabiegi z fungicydem opartym na miedzi, liczba zabiegów była adekwatna do momentu wykorzystania miedzi jak substancji aktywnej w dawce całkowitej do 3 kg Cu/ha/sezon. W trakcie całego sezonu prowadzono obserwacje dotyczące występowania objawów chorób (zaraza ziemniaka, alternarioza) powodujących zasychanie roślin. Efektywność zabiegów oceniano poprzez ocenę stopnia procentowego porażenia przez patogeny roślin ziemniaka w zależności od kombinacji doświadczenia. Stwierdzono, że *B. subtilis* wykazuje potencjał ochronny przed *P. infestans* w momencie prewencyjnego aplikowania, przy czym w warunkach polowych działanie zabezpieczające występuje jedynie po wykonaniu 6 zabiegów i jest porównywalne z zabiegami z miedzią.

Słowa kluczowe: bakterie pożyteczne, odmiany ziemniaka, prewencyjne zabiegi ochronne, zaraza ziemniaka

1. Introduction

This article discusses the research on the potential use of *Bacillus* bacteria [3] with a wide range of possibilities for crop protection. The use of antagonistic bacteria for some species of fungi has been common in domestic research for several years [8]. An anthropological impact of *Bacillus* spp. on many pathogenic fungi and fungal organisms has been described [10]. It has also been found that *B. subtilis* may be a growth promoter [4]. The presence of *B. subtilis* causes the death of pathogenic fungal cells or a significant decrease in their growth rate. In addition, it hampers the pathogen interaction with plant tissue by competing for rizo- and phyllosphere rats and nutrients. The presence of *B. subtilis* on the surface of plant organs induces reactions related to acquired plant immunity. It was found that *B. subtilis* showed higher efficacy in the protection of tomato against alternaria, less in case of late potato blight. The potential of this product can be used to protect organic crops, since it is a plant protection product already regis-

tered in Poland and it is possible to extend the scope of its use. In this study the influence of *B. subtilis* on the response of a plant defense system after potato infestation by *P. infestans* was evaluated. The aim of this study was to determine the effectiveness of different strategies of application with *B. subtilis* in order to ensure plants' healthiness.

2. Material and methods

Greenhouse tests

Serenade ASO is based on *B. subtilis*, it is registered and used to reduce potato pests on both tomatoes and potatoes crops in Chile, USA, New Zealand, Mexico, Japan, Israel, the Philippines, Guatemala, Honduras, Argentina, Italy, France, Turkey and others. In Europe Serenade ASO is registered in France, Italy, Switzerland and Germany for the limitation of bacterial diseases. Investigation on preventive and curative applications of Serenade ASO product after inoculation with *P. infestans* on potato plants under

greenhouse conditions were performed, potato plants at the stage BBCH 15 were placed individually in pots in a greenhouse. Inoculation with *P. infestans* and *B. subtilis* (beneficial bacterium) were applied at intervals of 3 days. Concentration of *P. infestans* (isolate 2295) was 1×10^6 /1ml (3ml per plant -in calculation 300l/water per 1 ha.). The interval between applications with *P. infestans* and *B. subtilis*, in other combination copper fungicide was applied as the first then after 3 days *P. infestans* solution was applied, etc. according to scheme shown in Fig.1. An assessment of the infestation was made twice: one week and two weeks after the first treatment using a percentage infestation of the plant according to EPPO recommendations.

Field research

Field trials were conducted at the IPP-NRI at the Experiment Station in Winna Góra (52.2N; 17.4E), where are located organic fields and in the private organic farm in Dąbrowa. Denar and Lord cv. were used, respectively. On each experimental field wheat was cultivated as a forecrop. The experiment was carried out on plots (size plot 100 m² with randomized distribution). As a preventive treatments Serenade ASO was applied as a spray at a dose of 8 l /ha and 3 and / or 6 treatments were made at intervals of 7 days. The first treatment (planned as preventive) was performed at stage BBCH 11. Symptoms of disease caused by *P. infestans*, *A. solani*, *A. alternata* were not observed on the plants in the field at the moment of beginning treatments. As a reference, copper-based fungicide treatments were used at total dose of 3 kg Cu/ha /season used as trade product Nordox. Three repetitions were made for each combination. Over the season, evaluation was made concerning the occurrence of disease symptoms (late potato blight, alternaria). Effectiveness of the treatments was assessed by assessing the percentage of infestation of potato plants, depending on the combination of experiments. In each combination 15 plants were marked and systematically

evaluated. Six rows, 50 m long, were included in one plot, with four replications. The presence of *P. infestans* spores in the field was confirmed in the laboratory. *Leptinotarsa decemlineata* was controlled by using the insecticide Biospin 120 SC (contains a.i. spinosad). In Dąbrowa, the observations began when symptoms were noted (13.07) and continued at one week intervals until the plants were dry. In Winna Góra (WG) the first evaluation was made on 19.07 and the second and the last on 26.07 (because of the high infestation of plant).

The results were subject to analysis of variance by means of the Statistica 9.1 program. A one-way ANOVA was carried out to determine effects of the different strategies of bacterium application, Tukey's test ($p = 0.05$) was performed to compare mean values. The results on figures are averaged from 15 plants from 2016.

3. Results and discussion

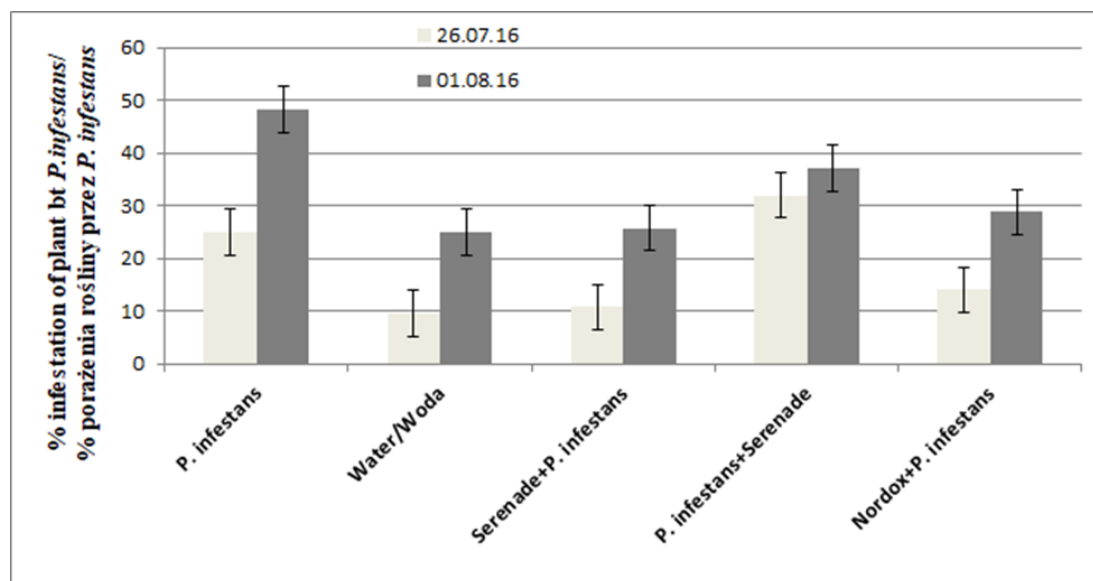
Investigation of preventive and interventional application of *P. infestans* on potato plants under greenhouse conditions

It has been found that *B. subtilis* has a protective potential when is used as preventive application, its effectiveness is similar to that with copper (Fig. 1). Also, the effect of water spraying is interesting, in this combination plant infestation by *P. infestans* was comparable to that of copper and the preventive use of *B. subtilis*. Perhaps water acts as an elicitor as well as mechanically rinses spores from plants without allowing them to penetrate plant cells.

Field trials

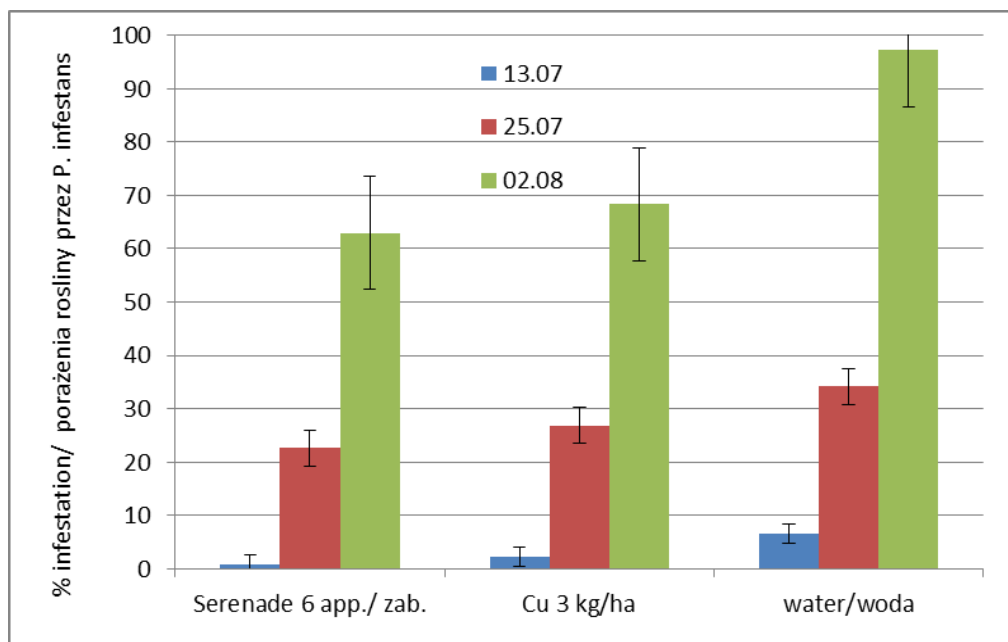
In the private farm, research was done on the Denar variety. The average percentage of pest infestation - the degree of plant infestation according to EPPO Standards - is shown in Fig. 2.

The results obtained for the Lord variety in Winna Góra (WG) are shown in Fig. 3.



Source: own work / Źródło: opracowanie własne

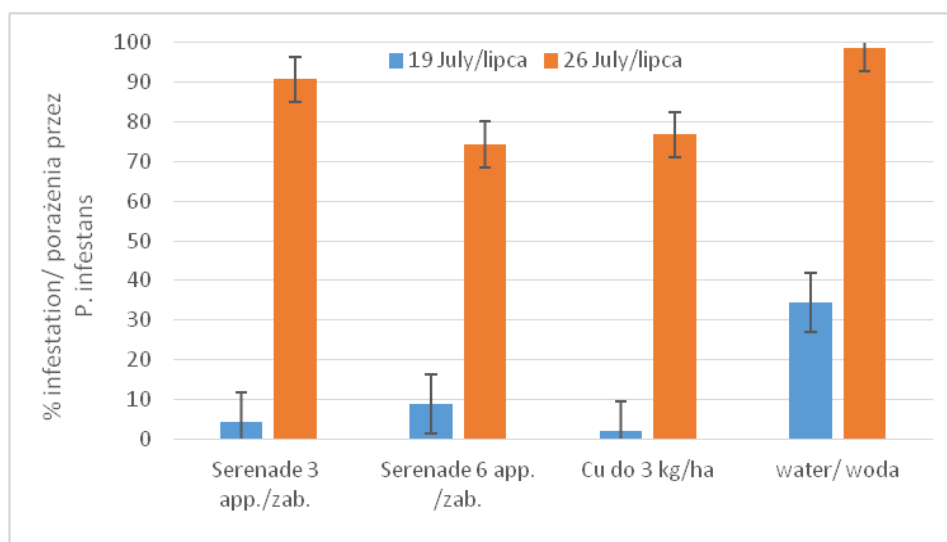
Fig. 1. Plant infestation (%) of potato depending on the date of inoculation with *P. infestans* and *B. subtilis* (the interval between applications with pathogen and *B. subtilis* was 3 days), means values \pm standard errors, pot experiment
Rys. 1. Porażenie (%) zarazą ziemniaka roślin ziemniaka w zależności od terminu sztucznej infekcji z *P. infestans* i aplikacji *B. subtilis* (odstęp pomiędzy dwoma aplikacjami wynosił 3 dni) – doświadczenie wazonowe, średnie \pm SE



Source: own work / Źródło: opracowanie własne

Fig. 2. Average percentage of plant infestation of Denar cv. depending on strategy and time of observation (1st assessment – on 13.07, 2nd assessment on 25.07, 3rd assessment on 02.08), means values \pm standard errors

Rys. 2. Średni procent zasychania roślin odm. Denar w zależności od strategii zabiegów i terminu obserwacji (I ocena – 13.07, II ocena 25.07, III ocena 02.08), średnia \pm SD



Source: own work / Źródło: opracowanie własne

Fig. 3. Average percentage of dryness of Lord (WG) plants depending on the strategy of the treatments and the date of observation (1st assessment – on 19.07, 2nd assessment on 26.07), means values \pm standard errors

Rys. 3. Średni procent zasychania roślin odm. Lord (WG) w zależności od strategii zabiegów i terminu obserwacji (I ocena – 19.07, II ocena 26.07), średnia \pm SD

Treatments with *B. subtilis*, six applications were more effective than only 3 applications. Effectiveness of six applications was similar like for copper treatments (total 4 applications) for both cultivars of potato (Fig. 2, 3).

B. subtilis (Ehrenberg), *Pseudomonas fluorescens* (Trevisan) and *P. aureofaciens* (Kluyver) are being applied against a variety of plant pathogens including, especially, damping-off and soft rots [1]. *B. subtilis*, a ubiquitous bacterium commonly found in various ecological niches that does not have any history of pathogenicity from contact in the environment, was shown to be a potential biocontrol agent of harmful phyto-pathogenic fungi and bacteria, mosquitoes and nematodes [6, 7].

A product called Serenade ASO based on *B. subtilis* shows different efficacy against potato blight, which may be due to the varied bacterial survival of the phyllosphere [6]. Therefore, there is a need to find a strategy (appropriate frequency of treatments) and to determine the conditions of use of *B. subtilis* as a preventive and / or protective agent. In Chowdappa et al. paper [3] the efficacy of *B. subtilis* OTPB1 and *Trichoderma harzianum* OPB3 was confirmed to inhibit the development of *Alternaria solani* and *Phytophthora infestans* and stimulation of growth and resistance of tomato plants. In the detached leaf assays described in the paper Stephan et al. (2005) the commercial preparations Elot-Vis, Serenade and Trichodex, and plant

extracts of *Rheum rhabarbarum* and *Solidago canadensis* showed a significant effect on the level of infestation by *P. infestans*. However, none of the treatments was as effective as copper. In the case of Serenade, the metabolites produced by its active microorganism, *B. subtilis*, were demonstrated to be the effective component of the formulation, and not the microorganism itself. In order to take curative and protective modes of action into account, the test substances were applied 24 h before, or 90 min after inoculation with *P. infestans*. Generally, better effects were obtained when the applications were made 24 h before inoculation. The optimum time of application of this bacterium is 72 before inoculation with *P. infestans*. It is with line with our results. Our research indicated that the formulation Serenade ASO can limit activation of the pathogen. We recommend continuing studies to confirm the effectiveness and usefulness of this bacterium in protection of potato in organic agricultural production.

4. Conclusion

It has been found that *B. subtilis* has the potential to protect against *P. infestans* when it was applied as preventive treatments, in field conditions the protective effect occurs only after 6 treatments and is comparable to that with copper.

5. References

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