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# The evaluation of *Pythium oligandrum* and chitosan in control of *Phytophthora infestans* (Mont.) de Bary on potato plants

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ABSTRACT

The studies were carried out both in *in vitro* and *in vivo* conditions. The field experiment was conducted during the years 2005-2007 at the Experimental Station in Mydlniki, owned by the Department of Plant Protection at the University of Agriculture in Krakow. The aim of these studies was to determine the effect of tuber dressing and plant spraying with Polyversum (B.A.S. *Pythium oligandrum*) and Biochikol 020 PC (B.A.S. chitosan) bio-preparations on the top leaves and tubers of 'Ibis' potatoes infested by *Phytophthora infestans*. The chemical preparation Vitavax 2000 FS (B.A.S. karboxin and thiuram) was used. The *in vitro* evaluation of Polyversum and Biochikol 020 PC bio-preparations on the *P. infestans* mycelium linear growth was investigated. The above-mentioned preparations were applied at three different concentrations. The experiment was carried out using the Kowalik and Krechniak method (1961). Based on the results it was found that the preparations under examination significantly inhibited top leaf

and tuber infestation by *Phytophthora infestans*. Moreover, according to the results obtained from *in vitro* tests, a significant effect from the Vitavax 2000 FS and Polyversum preparations and from the highest concentration (2%) of Biochikol 020 PC preparation on the percentage inhibition of *P. infestans* mycelium linear growth was observed (in comparison to the control).

## INTRODUCTION

Under the climatic conditions found in Poland, late blight (*Phytophthora infestans*) is the most dangerous disease affecting potato plants. Recently, destruction of potato crops has risen significantly (Kapsa 2004, Kurzawińska and Gajda 2004). Late blight is responsible for decreases in tuber quality and quantity. A decrease in yield is connected with the leaves' infestation by *P. infestans*. It leads to the inhibition of tuber development and to its infestation. The most frequent treatment in plant protection, including protection of potatoes, is the application of fungicides. It should be noted that there is a large range of fungicides recommended for potato protection against *P. infestans*. It is necessary to treat this plant with chemicals several times during the vegetation period. Taking this under consideration, research into the possibility of the replacement of synthetic fungicide with preparations based on natural substances or antagonistic organisms has been conducted. However, the increase in environmental contamination by pesticides has prompted the introduction of alternative methods to fight plant pathogens. In modern plant protection an increasing interest in biological methods that use antagonistic microorganisms to fight pathogens has been observed. Mycoparasite *Pythium oligandrum*, a biologically active substance of the bio-preparation Polyversum, belongs to the mentioned antagonistic organisms (Benhamou et al. 1997). Moreover, the application of chitosan as protection for many plants against diseases, especially as an immunity inducer for the plants, is highly promising (Lafontaine and Benhamou 1996, Bell et al. 1998, Orlikowski et al. 2002, Pięta et al. 2006, Nawrocki and Mazur 2007).

The objective of the present study was to assess the effect of potato tuber dressing and plant spraying with Polyversum and Biochikol 020 PC bio-preparations on the infestation of leaves and tubers by *P. infestans* during the vegetation period. In addition, the effect of the mentioned bio-preparations on the *P. infestans* mycelium linear growth was investigated under *in vitro* conditions.

## MATERIAL AND METHODS

The three-year (2005-2007) field experiment was conducted on brown soil at the Experimental Station in Mydlniki near Krakow in the Department of Plant

Protection, part of the University of Agriculture. During the investigations, potatoes of the 'Ibis' mid-early cultivar, which is susceptible to leaf infection and mid-resistant to tuber infection, were used.

Cultivation and fertilization of the soil were done according to proper agro-technique guidelines. The experiment was established in a space between 62.5 cm rows in the third 10 days of April. The method of random squares in four replications was applied and the experimental combinations were as follows: 1 – control – plants derived from no dressed tubers; 2 – plants derived from tubers dressed with Biochikol 020 PC (B.A.S. chitosan) at a concentration of 2.5%; 3 – plants derived from dressed tubers + plant spraying with Biochikol 020 PC at a concentration of 2.5%, four times; 4 – plants derived from tubers dressed with Polyversum (B.A.S. *Pythium oligandrum*) in a dose of 10 g $kg^{-1}$; 5 – plants derived from dressed tubers + plant spraying with Polyversum at a concentration of 0.05%, four times; 6 – plants derived from tubers dressed with Vitavax 2000 FS (B.A.S. karboxin and thiuram) in a dose of 5 ml $kg^{-1}$ .

The chemical sprayings were conducted with the use of a Kaskada sprayer (1000 l $ha^{-1}$). The first treatment with the use of Polyversum and Biochikol 020 PC bio-preparations was done when visible disease symptoms occurred on leaves. The next three sprayings were applied four times in each vegetation period in intervals of 10-14 days depending on weather conditions.

Disease severity was assessed visually beginning on the date of disease occurrence and based on a 1-9 point scale, where 1 – the lowest infestation, and 9 – the highest infestation (Roztropowicz 1985). Each time, 25 random plants from each experimental plot were individually inspected. In the final juxtaposition the mean degree of leaf infestation for each combination was calculated.

Crop yield was determined during harvest. From each plot the total crop was weighed and then average yield was calculated for each combination.

The intensity of potato tuber infection by *P. infestans* was assessed directly after harvest. The percentage of infested tubers in a sample was noted (100 tubers from each plot). The determined results were verified statistically using the analysis of variance followed by Duncan's test ($p = 0.05$).

The Kowalik and Krechniak (1961) method was used in the studies on the *in vitro* effect of preparations under consideration on the *P. infestans* mycelium linear growth. *P. infestans* was isolated from infected potato plants and cultivated on medium. This culture was used to further inoculations on media with the tested preparations. Each of the examined preparations were applied to the PDA medium at three different concentrations: Biochikol 020 PC – 0.5%, 1.0%, 2.0%; Polyversum – 0.05%, 0.1%, 0.2%; Vitavax 2000 FS – 0.025%, 0.05%, 0.1%. The test was carried out in five repetitions for each combination (one repetition – one

Petri dish). The Petri dishes with a PDA medium but without amendments were the control. The percentage inhibition of mycelium linear growth on media with amendments of a particular preparation compared to the growth of fungi on a control Petri dish was used to measure the preparation activity (Kowalik and Krechniak 1961). The obtained results were subjected to statistical analysis using analysis of variance. The multiple Duncan test was used for estimating the differences between mean values at a significance level of $p = 0.05$.

## RESULTS

The mean percentage of tubers infected by *P. infestans* (Tab. 1) was low (0.8-1.5). Field studies on late blight occurrence, conducted in Krakow-Mydlniki during the years 2005-2007, showed differences in the terms of the disease's appearance and its course. The course of meteorological conditions (rainfall and temperature), which had an influence on late blight development during the vegetation period, was presented in Table 2.

Table 1. Percentage of seed-tubers infested by *Phytophthora infestans*

| Year | | | Mean of years |
|---|---|---|---|
| 2005 | 2006 | 2007 | |
| 1.5 | 0.8 | 1.0 | 1.1 |

Table 2. Average temperatures and rainfall sums during the vegetation period in 2005-2007

| Year | Month | Average air temperature (°C) | | | | Rainfall sum (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10-day periods | | | Monthly | 10-day periods | | | Monthly |
| | | I | II | III | | I | II | III | |
| 2005 | May | 11.1 | 10.5 | 18.1 | 13.2 | 57.4 | 10.0 | 8.4 | 75.8 |
| | June | 12.5 | 17.1 | 18.2 | 15.9 | 44.0 | 18.8 | 4.0 | 66.8 |
| | July | 17.5 | 18.6 | 20.2 | 18.8 | 56.9 | 41.0 | 6.9 | 104.8 |
| | August | 16.0 | 16.5 | 18.1 | 16.9 | 59.8 | 28.5 | 12.3 | 100.6 |
| | September | 16.8 | 13.9 | 13.1 | 14.6 | 0.0 | 17.2 | 4.2 | 21.4 |
| 2006 | May | 12.5 | 14.3 | 14.0 | 13.6 | 8.5 | 7.8 | 25.7 | 42.0 |
| | June | 11.1 | 18.2 | 21.6 | 16.9 | 20.7 | 17.8 | 45.8 | 84.3 |
| | July | 19.7 | 20.5 | 22.7 | 20.9 | 3.8 | 2.6 | 18.2 | 24.6 |
| | August | 17.1 | 18.3 | 14.6 | 16.7 | 39.3 | 9.9 | 78.6 | 127.8 |
| | September | 15.4 | 15.9 | 14.8 | 15.4 | 16.6 | 0.0 | 31.5 | 48.1 |
| 2007 | May | 10.5 | 14.9 | 20.8 | 15.4 | 14.0 | 25.1 | 10.4 | 49.5 |
| | June | 18.7 | 18.8 | 17.1 | 18.2 | 21.3 | 10.2 | 32.2 | 63.7 |
| | July | 16.6 | 21.2 | 18.9 | 18.9 | 53.3 | 7.6 | 8.5 | 69.4 |
| | August | 18.7 | 19.0 | 17.9 | 18.5 | 1.2 | 48.3 | 16.2 | 65.7 |
| | September | 12.0 | 11.3 | 12.5 | 11.9 | 288.3 | 0.8 | 5.0 | 294.1 |

The late blight appeared in Krakow-Mydlniki on the 27th of June 2007, but the meteorological conditions caused the disease to develop slowly. In 2006, late blight occurred on the 5th of July, which was nine days later in comparison to 2007. The meteorological conditions were less favourable to the disease's progress (Tab. 2). In 2005 the first late blight symptoms were noted the latest – on the 10th of July. The meteorological conditions in 2005 were conducive to the disease's development in the Krakow region (Tab. 2). Throughout the experimental period the mean disease index of leaf infestation by *P. infestans*, in combinations where tested preparations were applied, was significantly lower than in the control (Tab. 3).

Table 3. The effect of tested preparations on halum infestation by *Phytophthora infestans*

| Combination | Mean degree of infestation in the 1-9 scale | | | |
|---|---|---|---|---|
| | 2005 | 2006 | 2007 | Mean |
| Control | 9.0 b* | 6.2 a | 9.0 b | 8.1 b |
| Tubers treated – Biochikol 020 PC | 8.0 a | 4.7 a | 7.6 a | 6.8 a |
| Tubers treated and plants sprayed – Biochikol 020 PC | 7.9 a | 4.7 a | 7.6 a | 6.8 a |
| Tubers treated – Polyversum | 7.8 a | 4.6 a | 7.7 a | 6.7 a |
| Tubers treated and plants sprayed – Polyversum | 7.7 a | 4.5 a | 7.5 a | 6.6 a |
| Tubers treated – Vitavax 2000 FS | 7.9 a | 4.7 a | 7.5 a | 6.7 a |

*Means in columns followed by the same letter do not differ at p = 0.05 according to multiple range Duncan's test

Tuber yield from the protected plots was higher than from the control (Fig. 1). The highest increase of yield was obtained in the combination where tubers were dressed and plants were sprayed with Biochikol 020 PC in every experimental year. Moreover, in 2006 the application of Vitavax 2000 FS to tuber dressings had a significant influence on the total yield increase, whereas in 2007 all applied preparations significantly increased total yield (Fig. 1). Based on the results from all analyses of tuber infestation by *P. infestans* made directly after harvesting, it was found that all preparations under examination contributed to a lower degree of tuber infestation by the pathogen. In all of the years of investigation the percent of tuber infestation by *P. infestans* was significantly lower in those combinations where tested preparations were used (Tab. 4).

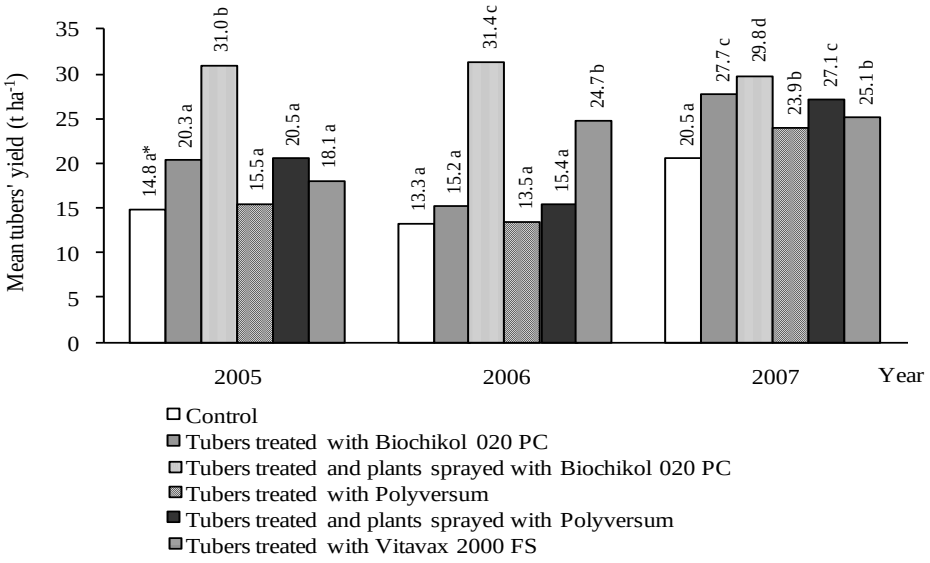



Figure 1. The effect of the tested preparations on potato yield

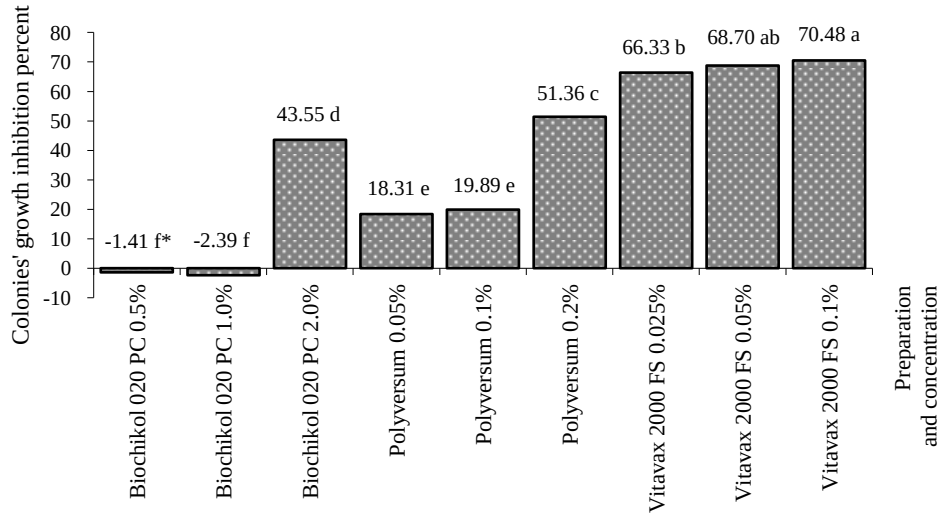
Table 4. The effect of tested preparations on tuber infestation by *Phytophthora infestans* (%)

| Combination | Year | | | |
|---|---|---|---|---|
| | 2005 | 2006 | 2007 | Mean |
| Control | 4.5 b* | 3.0 b | 4.6 b | 4.0 b |
| Tubers treated – Biochikol 020 PC | 2.5 a | 2.3 a | 2.5 a | 2.4 a |
| Tubers treated and plants sprayed – Biochikol 020 PC | 1.8 a | 1.7 a | 2.0 a | 1.8 a |
| Tubers treated – Polyversum | 2.5 a | 2.1 a | 2.3 a | 2.3 a |
| Tubers treated and plants sprayed – Polyversum | 2.0 a | 1.8 a | 1.8 a | 1.9 a |
| Tubers treated – Vitavax 2000 FS | 2.3 a | 2.1 a | 2.0 a | 2.1 a |

*Means in columns followed by the same letter do not differ at p = 0.05 according to multiple range Duncan's test

Figure 2 represents the percentage inhibition of *P. infestans* mycelium linear growth. Among the preparations under examination, Vitavax 2000 FS applied at all concentrations (0.025%, 0.05%, 0.1%) limited *P. infestans* mycelium linear growth the most. The percentage inhibition was 66.33%, 68.70%, and 70.48%,

respectively (Fig. 2). The inhibition of mycelium linear growth of the fungus was found also in combinations where bio-preparation Polyversum was used in all tested doses, but the best inhibition (51.36%) was noted at the highest concentration of this preparation, which was 0.2%. There were no statistically important differences recorded between concentrations of 0.1% and 0.05% (Fig. 2). The least *in vitro* anti-fungal activity was found in combinations where Biochikol 020 PC was used. Biochikol 020 PC significantly limited *P. infestans* mycelium linear growth only when applied at the highest concentration (2.0%), whereas in doses of 0.5% and 1.0% it showed a stimulating effect on the pathogen's mycelium linear growth (Fig. 2).




*Means followed by the same letter are not significantly different at p = 0.05 according to Duncan's multiple range test

Figure 2. The effectiveness of tested preparations in relation to *Phytophthora infestans*

According to statistical calculations, a significant influence of the preparations under examination (with the exception of Biochikol 020 PC at concentrations of 0.5% and 1.0%) on the percentage inhibition of *P. infestans* mycelium linear growth was noted.

## DISCUSSION

On the basis of the observations conducted in Krakow-Mydliniki during the years 2005-2007, it was noted that in addition to the immunity of potato plants,

*P. infestans* occurrence and growth depended on the meteorological conditions during the vegetation period.

Kapsa (2004) includes plentiful rainfall, high relative air humidity and long lasting leaf moistening to the most important factors that favour leaf infections by *P. infestans*. Results obtained in the three-year field experiments showed the possibility of the use of both Polyversum and Biochikol 020 PC in the protection of potatoes against late blight. The mean disease index of leaf infestation by *P. infestans* was significantly lower than in the control in combinations where the tested preparations were applied. The preparations had an influence on the significantly lower (in comparison to the control) percentage of tuber infestation by *P. infestans*. It is believed that the application of microbiological material on tuber surfaces is the most effective method in preventing infestation. According to Martin and Hancock (1987), *P. oligandrum* colonises the ecological niche in the soil and successfully competes with the plants' pathogens. The authors showed that *P. oligandrum* have an influence on pathogenic factors. When *P. oligandrum* is introduced in the soil, on seeds, tubers, bulbs, and rootstocks, a significant increase in plant health is noted. According to Deacon (1991), *P. oligandrum* demonstrates a destructive and parasitic character to many pathogenic fungi.

Bell et al. (1998), Pięta et al. (2006), Nawrocki and Mazur (2007) confirmed the efficiency of chitosan in vegetable protection. The results of the investigations conducted by the above-mentioned authors showed that the protective efficiency of chitosan applied as a means of prevention is clearly better than when applied as a means of intervention. The effectiveness of chitosan influence on phytopathogens depends on its concentration and the virulence of infectious factors. According to Pośpieszny (1997), chitosan induces the immunity of many plants through cell wall lignification, phytoalexin production and the synthesis of protein inhibitors. The treatment of cells with chitosan stimulates them to intensify the production of additional structures and accumulate phenol substances, which are harmful for fungi.

Throughout the experimental period, the tuber yield from the protected plots was higher than from the control plots. The higher yield obtained from the protected plots, compared with the control, is most likely connected with the infestation of aboveground plant parts by *P. infestans.* According to Van der Plank (1963) a reduction of 50-70% of the assimilation area of the leaves limited the yield increase and earlier infections led to higher rates of plant infection. Among the tested preparations under *in vitro* conditions, the chemical preparation Vitavax 2000 FS limited *P. infestans* mycelium linear growth the most. In combinations where the Polyversum bio-preparation was applied, the inhibition of *P. infestans* mycelium linear growth was found, but the best inhibition was noted at the highest concentration of this preparation.

The least inhibition of mycelium linear growth was found in combinations where Biochikol 020 PC was used. Biochikol 020 PC applied at the highest concentration limited *P. infestans* mycelium linear growth, whereas the two lower doses of the preparation showed a stimulating effect on *P. infestans* mycelium linear growth.

According to Orlikowski et al. (2002) most of the studies on the mechanism of chitosan activity to pathogenic fungi showed that this substance did not inhibit mycelium growth and spore germination under *in vitro* conditions. However, according to Pośpieszny (1997), under *in vitro* conditions chitosan inhibited the growth of several fungi and microbes but not all of them.

## CONCLUSIONS

1. The applied preparations (Biochikol 020 PC, Polyversum, Vitavax 2000 FS) significantly reduced the mean degree of halum and percentage of tuber infestation by *P. infestans*.
2. The tuber yield from the protected plots was higher, in comparison with the control.
3. Polyversum preparation, chemical preparation (Vitavax 2000 FS) and Biochikol 020 PC but applied only at the highest concentration (0.2%) significantly reduced mycelium linear growth of *P. infestans* during *in vitro* examinations.

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## ZASTOSOWANIE *PYTHIUM OLIGANDRUM* I CHITOZANU W ZWALCZANIU *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY ZIEMNIAKA

S t r e s z c z e n i e: Badania wykonano w warunkach *in vitro* i *in vivo*. Doświadczenia polowe przeprowadzono w latach 2005-2007 w Stacji Doświadczalnej Katedry Ochrony Roślin Kraków-Mydlniki. Badano wpływ zaprawiania sadzeniaków i opryskiwania roślin biopreparatem Polyversum (s.a. *Pythium oligandrum)* i Biochikolem 020 PC (s.a. chitozan) na porażenie zarówno naci, jak i bulw ziemniaka odmiany 'Ibis' przez *Phytophthora infestans*. Jako preparat chemiczny użyto Vitavax 2000 FS (s.a. karboksyna + tiuram). W doświadczeniach prowadzonych w warunkach *in vitro* badano wpływ Polyversum i Biochikolu 020 PC w trzech różnych stężeniach na wzrost liniowy plechy *P. infestans*. Badania te wykonano metodą zatrutych podłoży Kowalika i Krechniaka (1961). Wyniki doświadczeń polowych wykazały, że badane preparaty w sposób istotny ograniczyły porażenie naci i bulw przez *P. infestans*. Na podstawie wyników badań w warunkach *in vitro* stwierdzono istotny (w stosunku do kontroli) wpływ Vitavaxu 2000 FS, biopreparatu Polyversum i Biochikolu 020 PC tylko w stężeniu największym (2%) na procent zahamowania wzrostu liniowego plechy *P. infestans*.

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