

**The usefulness of chitosan and *Pythium oligandrum*
in potato tuber protection against
*Helminthosporium solani***

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ABSTRACT

The effect of tuber dressing and plant spraying with Biochikol 020 PC (B.A.S. chitosan) and with Polyversum bio-preparation (B.A.S. *Pythium oligandrum*) on the infestation of stored tubers by *Helminthosporium solani* was studied. Vitavax 2000 FS (B.A.S. karboxin and thiuram) was used as a standard fungicide. The effect of these preparations applied at three different concentrations on *H. solani* mycelium linear growth was investigated under *in vitro* conditions according to the Kowalik and Krechniak method (1961). The application of all tested preparations during potato vegetation resulted in both lower (in comparison to the control) mean infestation degree and lower percent of tubers infected by this pathogen. Results showed that all preparations inhibited *H. solani* mycelium linear growth.

INTRODUCTION

The potato is still an economically important crop. This plant is infected by many pathogens during its vegetation as well as during tuber storage. *Helminthosporium solani* (Dur. Mont.), the culprit of silver scurf, commonly occurs in Poland, causing potatoes' yield losses by market value reduction and increase of tuber mass losses (Lutomirska 1999, Kurzawińska 2002). Silver scurf develops mostly during storage. Changes that arise by pathogen growth take the form of silver, grey and shiny spots. In the case of the disease's intensification, spots can cover a tuber's entire surface (Lutomirska and Szutkowska 2004).

Kapsa et al. (1998) reported on the high harmfulness of *H. solani*. The tubers' peel separates as the result of tuber infestation by this fungus. Damaged peels have an effect on the increase of evaporation. Because of this, tubers wilt and dry out and the pulp becomes dark which causes a decrease in the value of the potato. Moreover, affected tubers germinate weakly or do not germinate at all, which results in yield losses.

There is not a unanimous opinion about *H. solani* wintering in plant residues in the soil. According to Hall (1996), the discussed fungus does not show an ability of wintering directly in the soil and to the production of effective inoculum sources. Whereas Rouse (1997) claims that this pathogen is capable of surviving in the soil between consecutive potato cultivations. The diseased tubers (Kurzawińska 1990, Rudkiewicz and Sikorski 1986), soil (Rouse 1997, Szutkowska 1999), and storage dust (Carnegie et al. 1996) are infection sources.

The objective of our study was to assess the effect of tuber dressing and plant spraying during the vegetation period with the Biochikol 020 PC (B.A.S. chitosan) preparation and Polyversum bio-preparation (containing *Pythium oligandrum* oospores) on stored tubers' infestation by *H. solani*. Moreover, in examinations conducted under *in vitro* conditions, the effect of the mentioned preparations applied at three different concentrations on *H. solani* mycelium linear growth was investigated.

MATERIAL AND METHODS

The field experiment was conducted on potatoes of a mid-early 'Ibis' cultivar at the Experimental Station at Mydlniki near Kraków in 2005 – 2007. Cultivation and fertilization were carried out according to recommendations of proper agro-technique. Directly before planting an assessment of the state of tuber health with regard to infestation by *H. solani* was made. The determination of the mean

infestation degree and proportional participation of diseased tubers in collected samples (100 tubers in four replications) was the base of the evaluation of the state of tuber health. The experiment was begun in the third week of April using the method of random squares in four replications (100 tubers on each plot) and in the following combinations: 1 – control – plants derived from tubers without any protection treatment; 2 – plants derived from tubers dressed with Biochikol 020 PC at a concentration of 2.5%; 3 – dressed tubers + plants sprayed four times with Biochikol 020 PC at a concentration of 2.5%; 4 – plants derived from tubers dressed with Polyversum in a dose of 10 g kg⁻¹ tubers; 5 – dressed tubers + plants sprayed four times with Polyversum at a concentration of 0.05%; 6 – plants derived from tubers dressed with Vitavax 2000 FS in a dose of 5 ml kg⁻¹ tubers. The first spraying with Biochikol 020 PC and Polyversum preparations was made after observation of the first *H. solani* symptoms on the haulm of the tested plants. The next three sprayings were carried out in 10-14 days, depending on weather conditions.

After harvesting, 100 tubers from each plot were randomly chosen, collected and put in storage. The analyses of tuber infestation by *H. solani* were made directly after potato harvesting (in September) and later every three months (in December and March).

In randomly chosen samples the share of diseased tubers was determined. The mean degree of tuber infestation by the aforementioned pathogen was defined on the base of a 1-9 scale, where 1 = the least infestation and 9 = very strong infestation (Roztropowicz 1985). The results were subjected to statistical analysis using analysis of variance. The multiple t-Duncan test was used for estimating the differences between mean values at significance level $p = 0.05$.

The Kowalik and Krechniak (1961) method was used to investigate the *in vitro* effect of the preparations on *H. solani* mycelium linear growth. *H. solani* was isolated from the tubers. Each of the examined preparations were applied at three concentrations: Biochikol 020 PC: 0.5%, 1.0%, 2.0%; Polyversum: 0.05%, 0.1%, 0.2%; Vitavax 2000 FS in doses: 0.025%, 0.05%, 0.1%. The test was carried out in five replications for each combination (10 Petri dishes for 1 repetition). The controls were Petri dishes with a PDA medium but without modifications. The percentage of inhibition of mycelium linear growth on the medium with modifications by the appropriate preparation compared to the growth of fungus on control Petri dishes was used to measure the preparations' activity (Borecki 1984). An estimate of significant differences between individual preparations and their concentrations was statistically analyzed using the multiple t-Duncan test.

RESULTS AND DISCUSSION

Both the mean degree and the percentage of tubers infected by *H. solani* were low and approximately came to 1.2-1.8; 4.2-5.5% (Table 1). Results received from three years of investigations show the favourable effect of tuber dressing and plant spraying with tested preparations on the reduction of silver scurf on descendant tubers (Table 2). According to results obtained from analyses of tuber infestation by *H. solani* made directly after harvesting (in September), 5.9% of tubers were infested in the control combination and only 1.9% of tubers in the combination where tubers were dressed with Vitavax 2000 FS (Table 2). The application of the chemical standard preparation (Vitavax 2000 FS) to the tuber dressing had the best effect on the inhibition of tubers' infestation degree, as shown by the tubers' infestation percentage directly after harvesting (Table 2). Among biological preparations taken under consideration, the Polyversum preparation applied to tuber dressing and plant spraying showed the best protective effect in this term of analysis (mean degree – 0.35; percent of affected tubers – 2.0) – Table 2.

Silver scurf belongs to one of the potato peel diseases, which develops during the storage period (Kapsa et al. 1998). The results of our investigations confirm this fact. After three and six months of tuber storage the increase of tuber infestation by *H. solani* and higher tuber infestation percentages was observed in all years of the experiment. The highest proportional infestation of tubers and the highest mean infestation degree by *H. solani* was found in the control combination after three and six months of tuber storage (Table 2). In March 32.7% tubers were infected and their infestation degree was 2.8. Statistical calculations showed that both the percentage of infected tubers and the mean infestation degree by *H. solani* in combinations with biological preparation application were considerably lower as compared to the control. Earlier (Kurzawińska 1990, Kurzawińska and Gajda 2004), it was found that many factors affect disease intensity: state of tuber health, quantity and quality of infection sources, applied protective treatment and the reaction of the potato variety to disease.

Figure 1 presents the efficiency of tested preparations in relation to *H. solani*. Among examined preparations, Vitavax 2000 FS applied at all concentrations (0.025%, 0.05%, 0.1%) limited the most mycelium linear growth of the discussed pathogen. The percent of inhibition approximately came to 84.3%, 89.6% and 99.7% (Fig. 1). In combinations where Biochikol 020 PC was used, the greatest inhibition percentage of *H. solani* colony growth was noted at the 2% concentration of the mentioned preparation (74.0). The weakest fungistatic activity under *in vitro* conditions was found in combinations with Polyversum bio-preparation. According to statistical calculations, a significant effect (in comparison to the control) of all preparations under consideration on proportional inhibition of *H. solani* colony growth was shown.

The conducted experiments showed the possibility of both chitosan and *P. oligandrum* use in potato protection against tuber infection by *H. solani*. According to Gajda and Kurzawińska (2004a, 2004b), *P. oligandrum* limits potato infestation by *H. solani* under *in vitro* as well as *in vivo* conditions. Strong chitosan efficiency in potato protection against fungal and peel pathogens was confirmed in investigations conducted by Kurzawińska and Mazur (2007). Results obtained from experimentation proved that the application of tested natural medications may protect potato tubers from silver scurf to a high degree. There is an opportunity for the application of the considered preparations in organic potato production.

Table 1. Estimation of state of tuber health with regard of infestation by *Helminthosporium solani*

Feature	Years			Mean
	2005	2006	2007	
Percent of infested tubers	4.2	4.9	5.5	4.9
Mean	1.2	1.6	1.8	1.5

Table 2. Influence of investigated preparations on occurrence of silver scurf (*Helminthosporium solani*) on tubers – means for 3 years

Combination	Mean degree of infestation at 1-9 scale			Percent of infested tubers		
	Date of analysis			Date of analysis		
	September	December	March	September	December	March
Control	1.20 c*	1.58 e	2.8 d	5.9 c	18.9 c	32.7 e
Tubers dressed – Biochikol 020 PC	0.45 ab	1.33 d	2.05 c	2.6 b	12.0 b	17.8 d
Tubers dressed and plants sprayed – Biochikol 020 PC	0.43 ab	1.23 bc	1.85 b	2.5 ab	10.1 a	14.9 b
Tubers dressed – Polyversum	0.60 b	1.28 cd	2.00 c	2.6 b	11.6 b	17.4 cd
Tubers dressed and plants sprayed – Polyversum	0.35 a	1.13 a	1.68 a	2.0 ab	9.9 a	13.9 a
Tubers dressed – Vitavax 2000 FS	0.33 a	1.15 ab	2.00 c	1.9 a	12.0 b	16.7 c

*values in the same column followed by the same letter do not differ at a 5% level of significance (Duncan's multiple range test)

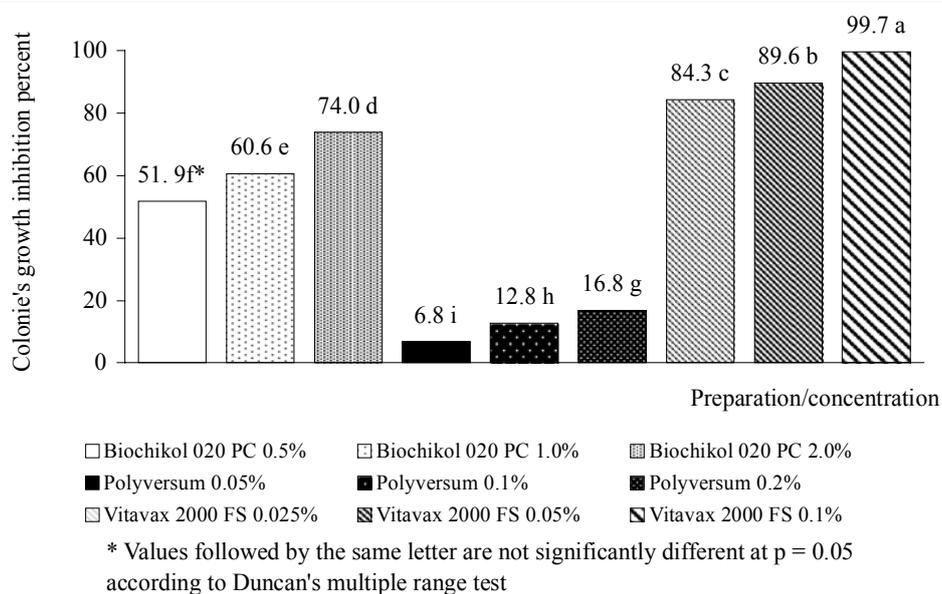


Fig. 1. The effectiveness of tested preparations in relation to *Helminthosporium solani*

CONCLUSIONS

1. During the potatoes' vegetation period, the applied preparations had an effect on lower tuber infestation by *H. solani* (in comparison to the control).
2. The best protective effect was noted in combinations with Polyversum and Biochikol 020 PC bio-preparations, applied to tuber dressing and sprayed on the plants four times.
3. Vitavax 200 FS (0.1%) and Biochikol 020 PC (2.0%) proved to be the most efficient in limiting *H. solani* mycelium linear growth.
4. Obtained results showed that *P. oligandrum* and chitosan could be useful in potato protection against *H. solani*.

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PRZYDATNOŚĆ CHITOZANU I *PYTHIUM OLIGANDRUM* W OCHRONIE BULW ZIEMNIAKA PRZED *HELMINTHOSPORIUM SOLANI*

Streszczenie: Celem 3-letnich badań polowych (2005 – 2007) było określenie wpływu zaprawiania sadzeniaków i opryskiwania roślin Biochikolem 020 PC (s.a. chitozan) i biopreparatem Polyversum (s.a. *Pythium oligandrum*) na porażenie przechowywanych bulw przez *Helminthosporium solani*. Jako preparat standardowy stosowano Vitavax 2000 FS (s.a. karboksyna + tiuram). W doświadczeniach prowadzonych w warunkach *in vitro* badano wpływ tych preparatów w trzech różnych stężeniach na wzrost liniowy grzybni *H. solani*.

Stosowane w okresie wegetacji ziemniaka preparaty wpłynęły na niższy (w stosunku do kontroli) stopień porażenia bulw przez *H. solani*. Procentowe porażenie bulw przez tego patogena w kombinacjach badanych preparatów było również istotnie niższe niż w kontroli.

Stwierdzono istotny (w stosunku do kontroli) wpływ badanych preparatów na procent zahamowania wzrostu kolonii *H. solani*. Reakcja *in vitro* zależała od rodzaju preparatu i jego stężenia.