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# BIOPRODUCTS IN CONTROL OF STRAWBERRY VERTICILLIUM WILT

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## Abstract

Remedier WP, which contains mycelium and spores of two antagonistic fungi (*Trichoderma harzianum* and *T. viride*), and three plant extracts (from pine and spruce needles, horsetail and seaweed, and from herbs) were tested under field conditions during four growing seasons to evaluate their ability to protect two strawberry cultivars ('Elsanta' or 'Honeoye') against *Verticillium dahliae*. Good results in reduction of wilt symptoms were observed after Remedier WP treatments. The product, used two times before planting, reduced the number of affected plants by 60% and was more effective than standard fungicide. Among tested extracts, seaweed and herbs were the most effective in wilt reduction (43 to 76% reduction, depending on disease severity). Other tested biological products were less effective (efficacy 35 to 60%, depending on disease severity).

**Key words:** strawberry, *Verticillium dahliae*, Verticillium wilt, biological control, *Trichoderma*, plant extracts

## Introduction

Verticillium wilt, caused by *Verticillium dahliae*, is a widespread disease that occurs in the most strawberry-production areas. The pathogen is soil-borne and infects plants through roots (Hanson 2000). It invades the plant's vascular system and prevents transport of water and nutrients (Kiraly et al. 1970). This is a polyphage and it can infect about 300 species of host plants, including many fruit plants, vegetables, forest trees, shrubs and flowers, as well as numerous weeds and some field crops. *Verticillium dahliae* survives in soil as microsclerotia which are produced in the dying tissues of the host plant. These structures can survive over a range of soil moisture and temperature conditions but loss of viability most rapidly in wet, warm soil (Uppal et al. 2008). Strawberry cultivars show different suscepti-

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bility to the disease. Some of them, such as 'Honeoye', 'Elsanta', 'Camarosa', 'Ventana', 'Diamante', 'Albion' and 'Kent' are very susceptible, while other, like: 'Senga Sengana', 'Dukat', 'Siabelle', 'Heros', 'Camino' do not show any wilt symptoms.

Yellowing, chlorosis and necrosis of lower leaves are usually the first symptoms of Verticillium wilt. Premature defoliation, most likely due to the drop in tissue turgor (Boote et al. 1983) and/or to the effect of *V. dahliae* phytotoxic compounds (Meyer et al. 1994) often occur. Younger leaves tend to remain green although stunted. Brownish streaks occur in vascular tissue of crown roots or at the base of the petiole. The losses can reach even 80% of plants under favorable environmental conditions. Control of the disease is very difficult, because of lack of effective fungicides. Only Topsin M 500 SC (thiofanate methyl) is recommended to control Verticillium wilt. Therefore, other methods, such as cultural practices, solarization, the use of tolerant cultivars and the alternatives to chemical pesticides in biological products are searched for (Uppal et al. 2008). Several studies have reported that some bacteria, fungi and plant extracts can serve as excellent biological control agents against soil-borne pathogens (Mercado-Blanco et al. 2004, Uppal et al. 2008).

The main purpose of this study was to determine biocontrol ability of selected plant extracts and bioproduct Remedier WP, which contains fungi *T. harzianum* and *T. viride*, against Verticillium wilt.

## Material and methods

Experiments were performed in Skierniewice during 2005–2008 seasons in the field with a podsol soil with clayey subsoil, where Verticillium wilt disease had been prevalent for many years. Before starting of the experiments, the number of *V. dahliae* propagules was on average 35 propagules per 1 g of the soil. It means that the risk of infection was very high in this field. One hundred plants (20 plants in each of five replicates per treatment) of strawberry cv. 'Honeoye' or 'Elsanta' (depending on the season), highly susceptible to Verticillium wilt, were planted. Tested plants were dipped or soil was sprayed with tested products (Remedier WP, which contains mycelium and spores of two antagonistic fungi *T. harzianum* and *T. viride*, and plant extracts solutions originating from pine and spruce needles, horsetail and seaweed or from herbs), one or two times before planting. The plant extracts were made by Agrobio Products (from bio-organic sources). Doses of the tested products are presented in Tables 1 and 2. In each season, strawberry stocks were planted in the beginning of June in spaces, 0.25 m within the rows and 1 m between rows. Non-chemical pesticides were used on experimental plots. Cultivation between rows was conducted during the growing season to control weeds.

After four months, plants were assessed for Verticillium wilt symptoms, using a six degree scale: 0 – no wilt symptoms, 1 – some chlorosis on the lower leaves, 2 – moderate necrosis of the lower leaves, 3 – severe leaf necrosis, 4 – chlorosis and necrosis on all leaves and plant decay symptoms, 5 – dead plant.

## Results

Remedier WP treatments could reduce symptoms of Verticillium wilt on strawberries under field conditions.

In 2006, Remedier WP significantly decreased the percentage of infected plants as compared to non-treated control ones, where 52% was affected with 2.1 degree of infestation. Remedier WP used at 0.4% concentration for dipping strawberry stocks significantly reduced the number of affected plants (effectiveness 64%). Additional watering of plants with this product, combination Remedier WP (2), did not have any positive influence on the effectiveness, which was 62.9%. Number of affected plants was the same as in the combination in which stocks were only dipped (Table 1 a). The second evaluated index in this experiment was the degree of plants infestation by *V. dahliae*. Remedier WP, in both combinations, reduced the disease intensity, by 66.7% and 76.2%, respectively. In both tested combinations Remedier WP was slightly superior to standard fungicide Topsin M 500 SC.

Good effectiveness of Remedier WP in reduction of strawberry Verticillium wilt was noted also in 2008 season. Remedier WP used at 2.5 kg/ha rate, two times for soil application before planting, decreased the number of affected plants by 59.8%. Its effectiveness was higher than that of standard fungicide Topsin M even by single application, seven days before or immediately before planting. The severity of disease on control plots was high – ca 60% of affected plants with an average infestation degree 1.7. Remedier WP used twice before planting reduced the disease intensity. Its effectiveness was 64.7% and it was a little higher than after one treatment with Remedier WP or with the standard fungicide Topsin M (Table 1 b).

The effect of plant extracts on Verticillium wilt was different. The best efficacy showed seaweed and herbs extracts. It reduced wilt incidence and disease severity by ca 43–70% and the degree of plant infestation by 55, 52 and 60%, depending on the season (Table 2). Extracts from horsetail or pine and spruce needles were less effective in control of strawberry Verticillium wilt. Their efficacy was about 30% (Table 2). Only in 2007 its effectiveness was higher (about 60%), which was probably connected with low disease pressure; in control plots only 31% of affected plants with 0.5 degree of infestation was observed.

## Discussion

Recently, as dessert strawberry cultivars became more and more popular in commercial production, Verticillium wilt has developed into great problem. Also banning fumigants such as methyl bromide from practical use and lack of cultural practices for effective disease control (Xiao et al. 1998), stimulate efforts to supplement chemical protection with new biological treatments (Hanson 2000). Many organisms, that occur naturally in the environment, have the ability to interfere with pathogens (Uppal et al. 2008). The field trials performed for over four years demonstrated that Remedier WP was effective in reduction of Verticillium

**Table 1**

Effectiveness of Remedier WP (*Trichoderma harzianum* and *T. viride*) in control of strawberry Verticillium wilt

a. Skierniewice – cv. 'Honeoye' – assessment: 11.10.2006

| Tested product | Concentration of the product (%) | Share of infected plants (%) | Effectiveness (%) | Degree of infestation (in 6 degree scale) | Effectiveness (%) |
|---|---|---|---|---|---|
| Untreated | – | 52.0 b | – | 2.1 b | – |
| Remedier WP (1), dipping plants | 0.4 | 18.7 a | 64.0 | 0.7 a | 66.7 |
| Remedier WP (2), dipping and watering plants | 0.4 | 19.3 a | 62.9 | 0.5 a | 76.2 |
| Topsin M 500 SC, standard | 0.2 | 31.4 ab | 39.6 | 1.1 a | 47.6 |

Means in columns followed by the same letter do not differ at 5% level of Newman-Keuls significance test at the 5% significance level.

b. Skierniewice – cv. 'Honeoye' – assessment: 25.09.2008

| Tested product | Concentration of the product or dose per 1 ha | Share of infected plants (%) | Effectiveness (%) | Degree of infestation (in 6 degree scale) | Effectiveness (%) |
|---|---|---|---|---|---|
| Untreated | – | 58.7 b | – | 1.7 b | – |
| Remedier, spraying the soil 2-times: 7 days and just before planting | 2.5 kg | 23.6 a | 59.8 | 0.6 a | 64.7 |
| Remedier, spraying the soil only 7 days before planting | 2.5 kg | 31.2 ab | 46.8 | 0.8 ab | 52.9 |
| Remedier, spraying the soil only just before planting | 2.5 kg | 32.8 ab | 44.1 | 0.9 ab | 47.1 |
| Topsin M 500 SC, dipping plants before planting | 0.2% | 40.4 ab | 31.2 | 1.0 ab | 41.2 |

Means in columns followed by the same letter do not differ at 5% level of Newman-Keuls significance test at the 5% significance level.

**Table 2**

Effectiveness of plant extracts in control of strawberry Verticillium wilt

| Tested product | Concentration of the product (%) | Share of infected plants (%) | Effectiveness (%) | Degree of infestation (in 6 degree scale) | Effectiveness (%) |
|---|---|---|---|---|---|
| Skierniewice – cv. 'Elsanta' – assessment: 9.09.2005 | | | | | |
| Untreated | – | 93.5 e | – | 2.0 c | – |
| Extract from pine and spruce needles, dipping plant roots before planting | 0.1 | 57.6 c | 38.4 | 1.3 b | 35.0 |
| Extract from seaweeds and herbs, dipping plant roots before planting | 1.0 | 53.1 b | 43.2 | 0.9 ab | 55.0 |
| Extract from horsetail, dipping plant roots before planting | 1.0 | 60.0 d | 35.8 | 1.1 b | 45.0 |
| Topsin M 500 SC, dipping plant roots before planting | 0.2 | 50.0 a | 46.5 | 0.5 a | 75.0 |
| Skierniewice – cv. 'Elsanta' – assessment: 19.09.2006 | | | | | |
| Untreated | – | 52.0 c | – | 2.1 b | – |
| Extract from pine and spruce needles, dipping plant roots before planting | 0.3 | 35.2 b | 32.3 | 1.5 a | 28.6 |
| Extract from seaweeds and herbs, dipping plant roots before planting | 2.0 | 21.0 a | 59.6 | 1.0 a | 52.4 |
| Extract from horsetail, dipping plant roots before planting | 2.0 | 36.7 b | 29.4 | 1.5 a | 28.6 |
| Topsin M 500 SC, dipping plants before planting | 0.2 | 31.4 b | 77.9 | 1.1 a | 47.6 |
| Skierniewice – cv. 'Elsanta' – assessment: 4.10.2007 | | | | | |
| Untreated | – | 31.2 b | – | 0.5 b | – |
| Extract from seaweeds and herbs, dipping plant roots before planting | 2.0 | 7.6 a | 75.6 | 0.2 a | 60.0 |
| Extract from horsetail, dipping plant roots before planting | 2.0 | 12.4 a | 60.3 | 0.2 a | 60.0 |
| Topsin M 500 SC, dipping plants before planting | 0.2 | 6.7 a | 78.5 | 0.1 a | 80.0 |

Means in columns followed by the same letter do not differ at 5% level of Newman-Keuls significance test at the 5% significance level.

wilt in very susceptible 'Honeoye' cultivar. Plant extracts tested on 'Elsanta' also provided good disease control. Hanson (2000) obtained similar results with seaweed and herbs extracts on cotton in greenhouse experiments.

*Trichoderma* species are known as inducers of systemic resistance in plants. Application of *T. harzianum* to bean roots reduced severity of foliar disease and grey mold, caused by *Botrytis cinerea* (De Meyer et al. 1998). Also biological control of *V. dahliae* in cotton with a mixture of lignin and *T. viride* (Azimkhodzbayeva and Ramasanova 1990, after Hanson 2000) and with *Gliocladium* species (Keinath et al. 1990) has been reported. This bioactivity of *Gliocladium* was attributed to its antagonism to the pathogen (Keinath et al. 1990, after Hanson 2000). In other works bacterial isolates and plant extracts (canola, rapeseed, seaweed and Canada milkvetch) were tested (Uppal et al. 2008). Canada milkvetch extract was the most effective one in reducing potato wilt.

Results obtained in this work are very promising, but further research is needed to assess and improve the formulation of biological products and to define the best method of their application.

## Streszczenie

BIOPREPARATY W OCHRONIE TRUSKAWKI PRZED WERTICYLIOZĄ

Badano możliwości zastosowania produktów biologicznych jako alternatywy w ograniczaniu werticyliozy truskawki, wywoływanej przez *Verticillium dahliae*. Patogen jest powszechnie występującym polifagiem, porażającym wiele roślin zarówno uprawnych, jak i dziko rosnących. Zimuje w formie mikrosklerocjów, które mogą przetrwać w glebie bez obecności gospodarza nawet do kilkunastu lat. Zwalczanie patogenów glebowych, w tym grzyba *V. dahliae*, jest bardzo trudne, a często wręcz niemożliwe, ze względu na brak zarejestrowanych, skutecznych środków ochrony. W przeprowadzonych w latach 2005–2008 badaniach uzyskano dobre efekty w ograniczaniu werticyliozy za pomocą niektórych preparatów biologicznych. Środek Remedier WP, zawierający grzybnię i zarodniki grzybów rodzaju *Trichoderma* (*T. harzianum* i *T. viride*), zastosowany dwukrotnie, w formie opryskiwania gleby przed wysadzeniem roślin lub moczenia sadzonek truskawki, istotnie ograniczał liczbę zamierających roślin. Na plantacji o wysokim stopniu skażenia gleby jego efektywność wyniosła powyżej 60% i była większa od skuteczności zabiegu moczenia sadzonek w roztworze fungicydu Topsin M 500 SC. Inne badane środki biologiczne, zawierające wyciągi z igieł sosny i świerka lub z roślin morskich albo ziół, wykazały mniejszą, zróżnicowaną w zależności od nasilenia choroby, efektywność działania.

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